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* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
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 NEWS  3  SEP 09   CA/CAplus records now contain indexing from 1907 to the
                   present
 NEWS  4  DEC 08   INPADOC: Legal Status data reloaded
 NEWS  5  SEP 29   DISSABS now available on STN
 NEWS  6  OCT 10   PCTFULL: Two new display fields added
 NEWS  7  OCT 21   BIOSIS file reloaded and enhanced
 NEWS  8  OCT 28   BIOSIS file segment of TOXCENTER reloaded and enhanced
 NEWS  9  NOV 24   MSDS-CCOHS file reloaded
 NEWS 10  DEC 08   CABA reloaded with left truncation
 NEWS 11  DEC 08   IMS file names changed
 NEWS 12  DEC 09   Experimental property data collected by CAS now available
                   in REGISTRY
 NEWS 13  DEC 09   STN Entry Date available for display in REGISTRY and CA/CAplus
 NEWS 14  DEC 17   DGENE: Two new display fields added
 NEWS 15  DEC 18   BIOTECHNO no longer updated
 NEWS 16  DEC 19   CROPU no longer updated; subscriber discount no longer
                   available
 NEWS 17  DEC 22   Additional INPI reactions and pre-1907 documents added to CAS
                   databases
 NEWS 18  DEC 22   IFIPAT/IFIUDB/IFICDB reloaded with new data and search fields
 NEWS 19  DEC 22   ABI-INFORM now available on STN
 NEWS 20  JAN 27   Source of Registration (SR) information in REGISTRY updated
                   and searchable
 NEWS 21  JAN 27   A new search aid, the Company Name Thesaurus, available in
                   CA/CAplus
 NEWS 22  FEB 05   German (DE) application and patent publication number format
                   changes

 NEWS EXPRESS  DECEMBER 28 CURRENT WINDOWS VERSION IS V7.00, CURRENT
               MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),
               AND CURRENT DISCOVER FILE IS DATED 23 SEPTEMBER 2003
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FILE 'HOME' ENTERED AT 15:35:18 ON 23 FEB 2004

=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'USPATFULL' ENTERED AT 15:35:57 ON 23 FEB 2004
CA INDEXING COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 19 Feb 2004 (20040219/PD)
FILE LAST UPDATED: 19 Feb 2004 (20040219/ED)
HIGHEST GRANTED PATENT NUMBER: US6694518
HIGHEST APPLICATION PUBLICATION NUMBER: US2004034897
CA INDEXING IS CURRENT THROUGH 19 Feb 2004 (20040219/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 19 Feb 2004 (20040219/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Dec 2003
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Dec 2003

>>>  USPAT2 is now available.  USPATFULL contains full text of the     <<<
>>>  original, i.e., the earliest published granted patents or         <<<
>>>  applications.  USPAT2 contains full text of the latest US         <<<
>>>  publications, starting in 2001, for the inventions covered in     <<<
>>>  USPATFULL.  A USPATFULL record contains not only the original     <<<
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>>>  published document but also a list of any subsequent              <<<
>>>  publications.  The publication number, patent kind code, and      <<<
>>>  publication date for all the US publications for an invention     <<<
>>>  are displayed in the PI (Patent Information) field of USPATFULL   <<<
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>>>  through the new cluster USPATALL.  Type FILE USPATALL to          <<<
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>>>  classifications, or claims, that may potentially change from      <<<
>>>  the earliest to the latest publication.                           <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> s yellow fever virus
        209552 YELLOW
         15811 FEVER
         70100 VIRUS
L1         661 YELLOW FEVER VIRUS
                (YELLOW(W)FEVER(W)VIRUS)

=> s l1 and (yellow fever virus/clm)
         12294 YELLOW/CLM
          1051 FEVER/CLM
         11930 VIRUS/CLM
            59 YELLOW FEVER VIRUS/CLM
                 ((YELLOW(W)FEVER(W)VIRUS)/CLM)
L2           59 L1 AND (YELLOW FEVER VIRUS/CLM)

=> s l2 and (prM and E)
          1268 PRM
       2292897 E
L3           17 L2 AND (PRM AND E)

=> s l3 and (prM/clm)
            44 PRM/CLM
L4           4 L3 AND (PRM/CLM)

=> d l4,cbib,1-4

L4   ANSWER 1 OF 4  USPATFULL on STN
2004:12950 Novel flavivirus antigens.
    Apt, Doris, Sunnyvale, CA, UNITED STATES
    Punnonen, Juha, Belmont, CA, UNITED STATES
    Brinkman, Alice M., Lake Bluff, IL, UNITED STATES
    Maxygen, Inc. Patent Department, Redwood City, CA, UNITED STATES (U.S.
    corporation)
    US 2004009469 A1 20040115
    APPLICATION: US 2003-375932  A1  20030226 (10)
    PRIORITY: US 2002-360030P  20020226 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 2 OF 4  USPATFULL on STN
2003:257279 Viral vaccine production method.
    Monath, Thomas P., Harvard, MA, UNITED STATES
    Guirakhoo, Farshad, Melrose, MA, UNITED STATES
    Arroyo, Juan, S. Weymouth, MA, UNITED STATES
    US 2003180329 A1 20030925
    APPLICATION: US 2003-342681  A1  20030115 (10)
    PRIORITY: US 2002-348565P  20020115 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 3 OF 4  USPATFULL on STN
2003:187415 Methods of preventing and treating flavivirus infection in animals.
    Monath, Thomas P., Harvard, MA, UNITED STATES
    Arroyo, Juan, S. Weymouth, MA, UNITED STATES
    US 2003129201 A1 20030710
    APPLICATION: US 2002-277478  A1  20021021 (10)
    PRIORITY: US 2001-346845P  20011019 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4   ANSWER 4 OF 4  USPATFULL on STN
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2003:64652 Chimeric flavivirus vectors.
Kleanthous, Harold, Westford, MA, UNITED STATES
Miller, Charles, Medford, MA, UNITED STATES
Oros, Larisa, Boston, MA, UNITED STATES
US 2003044773 A1 20030306
APPLICATION: US 2002-160939 A1 20020531 (10)
PRIORITY: US 2001-295265P 20010601 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d his

(FILE 'HOME' ENTERED AT 15:35:18 ON 23 FEB 2004)

FILE 'USPATFULL' ENTERED AT 15:35:57 ON 23 FEB 2004
L1 661 S YELLOW FEVER VIRUS
L2 59 S L1 AND (YELLOW FEVER VIRUS/CLM)
L3 17 S L2 AND (PRM AND E)
L4 4 S L3 AND (PRM/CLM)

=> s l3 not l4
L5 13 L3 NOT L4

=> s l3 and E/clm
402860 E/CLM
L6 7 L3 AND E/CLM

=> d l6,bib,1-7

L6 ANSWER 1 OF 7 USPATFULL on STN
Full Text
AN 2003:276776 USPATFULL
TI Use of flavivirus for the expression of protein epitopes and development of new live attenuated vaccine virus to immune against flavivirus and other infectious agents
IN Bonaldo, Mirna C., Rio de Janeiro, BRAZIL
Galler, Ricardo, Rio de Janeiro, BRAZIL
Freire, Marcos da Silva, Rio de Janeiro, BRAZIL
Garrat, Richard C., Sao Paulo, BRAZIL
PI US 2003194801 A1 20031016
AI US 2003-275707 A1 20030410 (10)
WO 2002-BR36 20020308
PRAI GB 2001-5877 20010309
DT Utility
FS APPLICATION
LREP NIXON & VANDERHYE, PC, 1100 N GLEBE ROAD, 8TH FLOOR, ARLINGTON, VA, 22201-4714
CLMN Number of Claims: 58
ECL Exemplary Claim: 1
DRWN 18 Drawing Page(s)
LN.CNT 3115
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 2 OF 7 USPATFULL on STN
Full Text
AN 2003:257279 USPATFULL
TI Viral vaccine production method
IN Monath, Thomas P., Harvard, MA, UNITED STATES
Guirakhoo, Farshad, Melrose, MA, UNITED STATES
Arroyo, Juan, S. Weymouth, MA, UNITED STATES
PI US 2003180329 A1 20030925
AI US 2003-342681 A1 20030115 (10)
PRAI US 2002-348565P 20020115 (60)
DT Utility
FS APPLICATION
LREP CLARK & ELBING LLP, 101 FEDERAL STREET, BOSTON, MA, 02110
CLMN Number of Claims: 15
ECL Exemplary Claim: 1
DRWN 9 Drawing Page(s)
LN.CNT 788
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 3 OF 7 USPATFULL on STN
Full Text
AN 2003:64652 USPATFULL
TI Chimeric flavivirus vectors
IN Kleanthous, Harold, Westford, MA, UNITED STATES
Miller, Charles, Medford, MA, UNITED STATES

Oros, Larisa, Boston, MA, UNITED STATES
PI US 2003044773 A1 20030306
AI US 2002-160939 A1 20020531 (10)
PRAI US 2001-295265P 20010601 (60)
DT Utility
FS APPLICATION
LREP CLARK & ELBING LLP, 101 FEDERAL STREET, BOSTON, MA, 02110
CLMN Number of Claims: 26
ECL Exemplary Claim: 1
DRWN 12 Drawing Page(s)
LN.CNT 753
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 4 OF 7 USPATFULL on STN
Full Text
AN 2003:30900 USPATFULL
TI Nucleic acid vaccines for prevention of flavivirus infection
IN Chang, Gwong-Jen J., Fort Collins, CO, UNITED STATES
PI US 2003022849 A1 20030130
AI US 2001-826115 A1 20010404 (9)
RLI Continuation-in-part of Ser. No. US 2001-701536, filed on 18 Jun 2001, PENDING A 371 of International Ser. No. WO 1999-US12298, filed on 3 Jun 1999, UNKNOWN
PRAI US 1998-87908P 19980604 (60)
DT Utility
FS APPLICATION
LREP Mary L. Miller, Esq., NEEDLE & ROSENBERG, P.C., The Candler Building, Suite 1200, 127 Peachtree Street, N.E., Atlanta, GA, 30303-1811
CLMN Number of Claims: 43
ECL Exemplary Claim: 1
DRWN 5 Drawing Page(s)
LN.CNT 4194
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 5 OF 7 USPATFULL on STN
Full Text
AN 2000:174106 USPATFULL
TI Subunit immonogenic composition against dengue infection
IN Ivy, John, Kailua, HI, United States
Nakano, Eilen, Hon., HI, United States
Clements, David, Honolulu, HI, United States
PA Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
PI US 6165477 20001226
AI US 1997-915152 19970820 (8)
RLI Continuation of Ser. No. US 1995-500469, filed on 10 Jul 1995, now abandoned which is a continuation-in-part of Ser. No. US 1995-488807, filed on 7 Jun 1995, now abandoned which is a continuation-in-part of Ser. No. US 1995-448734, filed on 24 May 1995, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Scheiner, Laurie; Assistant Examiner: Parkin, Jeffrey S.
LREP Morrison & Foerster LLP
CLMN Number of Claims: 13
ECL Exemplary Claim: 4,9
DRWN 19 Drawing Figure(s); 19 Drawing Page(s)
LN.CNT 4059
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 6 OF 7 USPATFULL on STN
Full Text
AN 2000:142128 USPATFULL
TI Methods of preparing carboxy-terminally truncated recombinant flavivirus envelope glycoproteins employing drosophila melanogaster expression systems
IN Ivy, John, Kailua, HI, United States
Nakano, Eilen, Honolulu, HI, United States
Clements, David, Honolulu, HI, United States
PA Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
PI US 6136561 20001024
AI US 1997-937195 19970925 (8)
RLI Continuation of Ser. No. US 1995-488807, filed on 8 Jun 1995 which is a continuation-in-part of Ser. No. US 1995-488734, filed on 24 May 1995
DT Utility
FS Granted
EXNAM Primary Examiner: Scheiner, Laurie; Assistant Examiner: Parkin, Jeffrey S.

LREP Morrison & Foerster, LLP
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 18 Drawing Figure(s); 18 Drawing Page(s)
LN.CNT 2450
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 7 OF 7 USPATFULL on STN
Full Text
AN 1998:44886 USPATFULL
TI Flavivirus recombinant poxvirus immunological composition
IN Paoletti, Enzo, Delmar, NY, United States
Pincus, Steven Elliot, East Greenbush, NY, United States
PA Virogenetics Corporation, Troy, NY, United States (U.S. corporation)
PI US 5744141 19980428
AI US 1995-484304 19950607 (8)
RLI Division of Ser. No. US 1994-224391, filed on 7 Apr 1994, now patented, Pat. No. US 5744140 And a continuation-in-part of Ser. No. US 1993-105483, filed on 12 Aug 1993, now patented, Pat. No. US 5494807 which is a continuation of Ser. No. US 1992-847951, filed on 6 Mar 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-713967, filed on 11 Jun 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-666056, filed on 7 Mar 1991, now abandoned , said Ser. No. US -224391 which is a continuation of Ser. No. US 1991-729800, filed on 17 Jul 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-714687, filed on 13 Jun 1991, now patented, Pat. No. US 5514375 which is a continuation-in-part of Ser. No. US 1991-711429, filed on 6 Jun 1991, now abandoned which is a continuation-in-part of Ser. No. US 1990-567960, filed on 15 Aug 1990, now abandoned , said Ser. No. US -714687 which is a continuation-in-part of Ser. No. US 1991-713967, filed on 11 Jun 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-666056, filed on 7 Mar 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Knode, Marian C.; Assistant Examiner: Salimi, Ali
LREP Frommer Lawrence & Haug LLP, Frommer, William S., Kowalski, Thomas J.
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN 27 Drawing Figure(s); 24 Drawing Page(s)
LN.CNT 3301
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l6,cbib,1-7

L6 ANSWER 1 OF 7 USPATFULL on STN
2003:276776 Use of flavivirus for the expression of protein epitopes and development of new live attenuated vaccine virus to immune against flavivirus and other infectious agents.
Bonaldo, Mirna C., Rio de Janeiro, BRAZIL
Galler, Ricardo, Rio de Janeiro, BRAZIL
Freire, Marcos da Silva, Rio de Janeiro, BRAZIL
Garrat, Richard C., Sao Paulo, BRAZIL
US 2003194801 A1 20031016
APPLICATION: US 2003-275707 A1 20030410 (10)
WO 2002-BR36 20020308
PRIORITY: GB 2001-5877 20010309
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 2 OF 7 USPATFULL on STN
2003:257279 Viral vaccine production method.
Monath, Thomas P., Harvard, MA, UNITED STATES
Guirakhoo, Farshad, Melrose, MA, UNITED STATES
Arroyo, Juan, S. Weymouth, MA, UNITED STATES
US 2003180329 A1 20030925
APPLICATION: US 2003-342681 A1 20030115 (10)
PRIORITY: US 2002-348565P 20020115 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 3 OF 7 USPATFULL on STN
2003:64652 Chimeric flavivirus vectors.
Kleanthous, Harold, Westford, MA, UNITED STATES
Miller, Charles, Medford, MA, UNITED STATES
Oros, Larisa, Boston, MA, UNITED STATES
US 2003044773 A1 20030306
APPLICATION: US 2002-160939 A1 20020531 (10)

PRIORITY: US 2001-295265P 20010601 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 4 OF 7 USPATFULL on STN
2003:30900 Nucleic acid vaccines for prevention of flavivirus infection.
Chang, Gwong-Jen J., Fort Collins, CO, UNITED STATES
US 2003022849 A1 20030130
APPLICATION: US 2001-826115 A1 20010404 (9)
PRIORITY: US 1998-87908P 19980604 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 5 OF 7 USPATFULL on STN
2000:174106 Subunit immonogenic composition against dengue infection.
Ivy, John, Kailua, HI, United States
Nakano, Eilen, Hon., HI, United States
Clements, David, Honolulu, HI, United States
Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
US 6165477 20001226
APPLICATION: US 1997-915152 19970820 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 6 OF 7 USPATFULL on STN
2000:142128 Methods of preparing carboxy-terminally truncated recombinant flavivirus envelope glycoproteins employing drosophila melanogaster expression systems.
Ivy, John, Kailua, HI, United States
Nakano, Eilen, Honolulu, HI, United States
Clements, David, Honolulu, HI, United States
Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
US 6136561 20001024
APPLICATION: US 1997-937195 19970925 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 7 OF 7 USPATFULL on STN
1998:44886 Flavivirus recombinant poxvirus immunological composition.
Paoletti, Enzo, Delmar, NY, United States
Pincus, Steven Elliot, East Greenbush, NY, United States
Virogenetics Corporation, Troy, NY, United States (U.S. corporation)
US 5744141 19980428
APPLICATION: US 1995-484304 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l6,kwic,7

L6 ANSWER 7 OF 7 USPATFULL on STN
AB . . . poxvirus, such as vaccinia virus, fowlpox virus and canarypox virus, containing foreign DNA from flavivirus, such as Japanese encephalitis virus, **yellow fever virus** and Dengue virus. In a preferred embodiment, the recombinant poxvirus generates an extracellular particle containing flavivirus **E** and M proteins capable of inducing neutralizing antibodies, hemagglutination-inhibiting antibodies and protective immunity against flavivirus infection. What is also described. . .
SUMM . . . sequence to be inserted into the virus, particularly an open reading frame from a non-pox source, is placed into an **E**. coli plasmid construct into which DNA homologous to a section of DNA of the poxvirus has been inserted.
SUMM . . . flanking a region of pox DNA containing a nonessential locus. The resulting plasmid construct is then amplified by growth within **E**. coli bacteria (Clewell, 1972) and isolated (Clewell et al., 1969; Maniatis et al., 1986).
SUMM Second, the isolated plasmid containing the DNA gene sequence to be inserted is transfected into a cell culture, **e**.g. chick embryo fibroblasts, along with the poxvirus. Recombination between homologous pox DNA in the plasmid and the viral genome respectively. . .
SUMM . . . the nonstructural glycoprotein NS1 and the remaining nonstructural proteins (Rice et al., 1985). The flavivirus virion contains an envelope glycoprotein, **E**, a membrane protein, M, and a capsid protein, C. In the case of Japanese encephalitis virus (JEV), virion preparations usually contain a small amount of the glycoprotein precursor to the membrane protein, **prM** (Mason et al., 1987a). Within

JEV-infected cells, on the other hand, the M protein is present almost exclusively as the higher molecular weight **prM** protein (Mason et al., 1987a; Shapiro et al., 1972).

SUMM . . . that have examined the protective effect of passively administered monoclonal antibodies (MAbs) specific for each of the three flavivirus glycoproteins (**prM, E,** NS1) have demonstrated that immunity to each of these antigens results in partial or complete protection from lethal viral challenge. Monoclonal antibodies to **E** can provide protection from infection by Japanese encephalitis virus (JEV) (Kimura-Kuroda et al., 1988; Mason et al., 1989), dengue type 2 virus (Kaufman et al., 1987) and **yellow fever virus** (YF) (Gould et al., 1986). In most cases, passive protection has been correlated with the ability of these **E** MAbs to neutralize the virus in vitro. Recently, Kaufman et al. (1989) have demonstrated that passive protection can also be produced with **prM** MAbs that exhibit weak or undetectable neutralizing activity in vitro. The ability of structural protein specific MAbs to protect animals. . . attenuate viral infection by blocking virus binding to target cells. Passive protection experiments using MAbs to the NS1 protein of **yellow fever virus** (Schlesinger et al., 1985; Gould et al., 1986) and dengue type 2 virus (Henchal et al., 1988) have demonstrated that. . .

SUMM . . . of NS1 immunity to protect the host from infection comes from direct immunization experiments in which NS1 purified from either **yellow fever virus**-infected cells (Schlesinger et al., 1985, 1986) or dengue type 2 virus-infected cells (Schlesinger et al., 1987) induced protective immunity from. . .

SUMM . . . NS1-based vaccines, dimerization of NS1 (Winkler et al., 1988) may be required to elicit the maximum protective response. For the **E** protein, correct: folding is probably required for eliciting a protective immune response since **E** protein antigens produced in **E.** coli (Mason et al., 1989) and the authentic **E** protein prepared under denaturing conditions (Wengler et al., 1989b) failed to induce neutralizing antibodies. Correct folding of the **E** protein may require the coordinated synthesis of the **prM** protein, since these proteins are found in heterodimers in the cell-associated forms of West Nile virus (Wengler et al., 1989a). The proper folding of **E** and the assembly of **E** and **prM** into viral particles may require the coordinated synthesis of the NS1 protein, which is coretained in an early compartment of the secretory apparatus along with immature forms of **E** in JEV-infected cells (Mason, 1989).

SUMM . . . a vaccinia recombinant containing the region of JEV encoding 65 out of the 127 amino acids of C, all of **prM,** all of **E,** and 59 out of the 352 amino acids of NS1. Haishi et al. (1989) reported a vaccinia recombinant containing Japanese encephalitis sequences encoding 17 out of the 167 amino acids of **prM,** all of **E** and 57 out of the 352 amino acids of NS1.

SUMM Deubel et al. (1988) reported a vaccinia recombinant containing the dengue-2 coding sequences for all of C, all of **prM,** all of **E** and 16 out of the 352 amino acids of NS1.

SUMM Zhao et al. (1987) reported a vaccinia recombinant containing the dengue-4 coding sequences for all of C, all of **prM,** all of **E,** all of NS1, and all of NS2A. Bray et al. (1989) reported a series of vaccinia recombinants containing the dengue-4 coding sequences for (i) all of C, all of **prM** and 416 out of the 454 amino acids of **E,** (ii) 15 out of the 167 amino acids of **prM** and 416 out of the 454 amino acids of **E,** (iii) 18 amino acids of influenza A virus hemagglutinin and 416 out of the 454 amino acids of **E,** and (iv) 71 amino acids of respiratory syncytial virus G glycoprotein and 416 out of the 454 amino acids of **E.**

SUMM Despite these attempts to produce recombinant flavivirus vaccines, the proper expression of the JEV **E** protein by the vaccinia recombinants has not been satisfactorily obtained. Although Haishi et al. (1989) demonstrated cytoplasmic expression of JEV **E** protein by their vaccinia recombinant, the distribution was different from that observed in JEV infected cells. Yasuda et al. (1990) detected expression of JEV **E** protein by their vaccinia recombinant on the cell surface. Recombinant viruses that express the **prM** and **E** protein protected mice from approximately 10 LD_{50} of challenge virus. Yasuda et al. (1990) elicited anti-JEV immune responses as well as protection but reactivity to a panel of **E** specific monoclonal antibodies exhibited differences from the reactivity observed in JEV infected cells.

SUMM . . . the viral ORF extending from C to NS2A under the control of the P7.5 early-late promoter produced intracellular forms of **prM, E,** and NS1 but failed to induce the synthesis of extracellular forms of any of the structural proteins, even though a. . . (Bray et al., 1989; Zhao et al., 1987). Additional recombinant viruses that contained several forms of the dengue type 4 **E** gene with or without other structural protein genes have also been examined (Bray et al., 1989). Although several of these recombinant viruses were able to induce protection, they neither produced extracellular forms of **E** nor induced

neutralizing antibodies. A dengue-vaccinia recombinant expressing a C-terminally truncated **E** protein gene induced the synthesis of an extracellular form of **E** and provided an increasing level of resistance to dengue virus encephalitis in inoculated mice (Men et al., 1991).

SUMM . . . protein capable of inducing protective immunity against flavivirus infection. In particular, the recombinant poxvirus generates an extracellular particle containing flavivirus **E** and M proteins capable of eliciting neutralizing antibodies and hemagglutination-inhibiting antibodies. The poxvirus is advantageously a vaccinia virus or an avipox virus, such as fowlpox virus or canarypox virus. The flavivirus is advantageously Japanese encephalitis virus, **yellow fever virus** and Dengue virus.

SUMM . . . In particular, the DNA contains Japanese encephalitis virus coding sequences that encode a precursor to structural protein M, structural protein **E**, and nonstructural proteins NS1 and NS2A. More in particular, the recombinant poxvirus contains therein DNA from flavivirus in a nonessential region of the poxvirus genome for expressing a particle containing flavivirus structural protein **E** and structural protein M.

SUMM More in particular, the recombinant viruses express portions of the flavivirus ORF extending from **prM** to NS2B. Biochemical analysis of cells infected with the recombinant viruses showed that the recombinant viruses specify the production of properly processed forms of all three flavivirus glycoproteins--**prM**, **E**, and NS1. The recombinant viruses induced synthesis of extracellular particles that contained fully processed forms of the M and **E** proteins. Furthermore, the results of mouse immunization studies demonstrated that the induction of neutralizing antibodies and high levels of protection. . .

DRWD FIG. 7 shows a comparison by SDS-PAGE analysis of the cell lysate **E** proteins produced by JEV infection and infection with the recombinant vaccinia viruses vP650, vP555, vP658 and vP583;

DRWD FIG. 8 shows a comparison by SDS-PAGE analysis of the culture fluid **E** proteins produced by JEV infection and infection with the recombinant vaccinia viruses vP650, vP555, vP658 and vP583;

DRWD FIG. 9 shows a comparison by sucrose gradient analysis of the forms of the **E** protein found in the culture fluid harvested from JEV infected cells and cells infected with vaccinia recombinants vP555 and vP650;

DETD . . . and B (SEQ ID NO:52) which contains the sequence of the C coding region combined with an updated sequence of **prM**, **E**, NS1, NS2A and NS2B coding regions.

DETD . . . The resulting plasmid, pJEV1, contained the viral ORF extending from the SacI site (nucleotide 2125) in the last third of **E** through the BalI site (nucleotide 4125) two amino acid residues (aa) into the predicted N terminus of NS2B (FIG. 1).

DETD . . . containing a XhoI sticky end, a SmaI site, the last 15 aa of C, and first 9 aa of JEV **prM** with a sticky HindIII end) were ligated to a HindIII-SacI fragment of JEV CDNA (nucleotides 407-2124), and XhoI-SacI digested vector. . . the viral ORF extending between the methionine (Met) codon (nucleotides 337-339) occurring 15 aa preceding the predicted N terminus of **prM** and the SacI site (nucleotide 2124) found in the last third of **E** (FIG. 1).

DETD . . . pJEV5, contained the viral ORF extending between the Met codon (nucleotides 811-813) occurring 25 aa preceding the N terminus of **E** and the SacI site (nucleotide 2124) found in the last third of **E** (FIG. 1).

DETD . . . (Kunkel, 1985) was used to change a potential vaccinia virus early transcription termination signal (Yuen et al., 1987) in the **E** gene of pJEV2 (TTTTTGT; nucleotides 1304-1310) to TCTTTGT, creating plasmid pJEV22 (FIG. 2). The same change was performed on pJEV5. . .

DETD . . . resulting plasmid, pJEV7, contained the viral ORF extending between the SacI site (nucleotide 2125) found in the last third of **E** and the last codon of NS2B (nucleotide 4512) (FIG. 2). SmaI-EagI digested pTP15 was purified and ligated to the purified. . .

DETD Four different vaccinia virus recombinants were constructed that expressed portions of the JEV coding region extending from **prM** through NS2B. The JEV cDNA sequences contained in these recombinant viruses are shown in FIG. 4. In all four recombinant. . .

DETD Recombinant vP555 encodes the putative 15 aa signal sequence preceding the N terminus of the structural protein precursor **prM**, the structural glycoprotein **E**, the nonstructural glycoprotein NS1, and the nonstructural protein NS2A (McAda et al., 1987). Recombinant vP583 encodes the putative signal sequence preceding the N terminus of **E**, **E**, NS1, and NS2A (McAda et al., 1987). Recombinant vP650 contains a cDNA encoding the same proteins as vP555 with the. . . vP583 with the addition of NS2B. In recombinants vP650 and vP658, a potential vaccinia virus early transcription termination signal in **E** (TTTTTGT; nucleotides 1087-1094) was modified to TCTTTGT without altering the aa sequence. This change was made in an attempt to increase the level of expression of **E** and NS1, since this sequence has been shown to

increase transcription termination in in vitro transcription assays (Yuen et al.,. . .

DETD . . . production by all four recombinants, suggesting that the potential vaccinia early transcriptional termination signal present near the end of the **E** coding region in vP555 and vP583 did not significantly reduce the amount of NS1 produced relative to vP650 or vP658. . .

DETD **E** and **prM** were Properly Processed when Expressed by Recombinant Vaccinia Viruses

DETD FIGS. 7 and 8 show a comparison of the **E** protein produced by JEV infection or infection with the recombinant vaccinia viruses. BHK cells were infected with JEV or recombinant. . .

DETD The data from the pulse-chase experiments depicted in FIGS. 7 and 8 demonstrate that proteins identical in size to **E** were synthesized in cells infected with all recombinant vaccinia viruses containing the **E** gene. However, the **E** protein was only released from cells infected with vaccinia viruses that contained the region of the viral ORF encoding **prM, E,** NS1, and NS2A (vP555 and vP650; see FIGS. 4, 7 and 8). Endoglycosidase sensitivity (FIGS. 7 and 8) revealed that both the intracellular and extracellular forms of the **E** protein synthesized by cells infected with the vaccinia recombinants were glycosylated; the cell-associated forms of **E** were endo H sensitive, whereas the extracellular forms were resistant to endo H digestion.

DETD Immunoprecipitates prepared from radiolabeled vaccinia-infected cells using a MAb specific for M (and **prM**) revealed that **prM** was synthesized in cells infected with vP555 and vP650. Cells infected with either of these recombinant vaccinia viruses produced cellular forms of **prM** that were identical in size to the **prM** protein produced by JEV-infected cells, and a M protein of the correct size was detected in the culture fluid of. . .

DETD The extracellular fluid harvested from cells infected with vP555 and vP650 contained forms of **E** that migrated with a peak of hemagglutinating activity in sucrose density gradients. Interestingly, this hemagglutinin migrated similarly to the slowly. . .

DETD Recombinant vaccinia virus vP555 produced **E**- and M-containing extracellular particles that behaved like empty viral envelopes. The ability of this recombinant virus to induce the synthesis. . .

DETD . . . described herein contain portions of the JEV ORF that encode the precursor to the structural protein M, the structural protein **E,** and nonstructural proteins NS1, NS2A, and NS2B. The **E** and NS1 proteins produced by cells infected with these recombinant viruses underwent proteolytic cleavage and N-linked carbohydrate addition in a. . . proteins produced by cells infected with JEV. These data further demonstrate that the proteolytic cleavage and N-linked carbohydrate addition to **E** and NS1 do not require flavivirus nonstructural proteins located 3' to NS2A in the viral genome (Bray et al., 1989;. . .

DETD . . . the portion of the ORF inserted in the recombinant vaccinia viruses had a significant effect on the late-stage processing of **prM** and **E,** but not on the fate of NS1. All recombinant viruses that encoded NS1 produced mature extracellular forms of this protein,. . . from transfected cells (Fan et al., 1990). On the other hand, only two of the four recombinants that contained the **E** protein coding region produced extracellular forms of **E**. These two recombinants, vP555 and vP650, differed from the remaining recombinants in that they contained the **prM** coding region in addition to **E,** NS1, and NS2A. The findings that extracellular forms of **E** were produced only by viruses containing the coding regions for both **E** and **prM** and that the extracellular forms of **E** were associated with M suggest that the simultaneous synthesis of **prM** and **E** is a requirement for the formation of particles that are targeted for the extracellular fluid.

DETD . . . the co-migration of the rabbit immunoglobulin heavy chain with the radiolabeled viral antigens, and to permit clear separation of the **E** and the NS1' proteins. Neutralization tests were performed on heat-inactivated sera (20 min. at 56° C.) as described (Tesh et. . .

DETD . . . virus, two viruses (vP555 and vP658) were selected for in-depth challenge studies. vP555 induced the synthesis of extracellular forms of **E,** whereas vP658 did not produce any extracellular forms of **E,** but contained additional cDNA sequences encoding the NS2B protein. In the challenge experiments several dilutions of challenge virus were tested,. . . dose of JEV. The analysis demonstrated that: (1) only those animals immunized with vP555 showed a strong immune response to **E,** and (2) a second inoculation resulted in a significant increase in reactivity to the **E** protein (FIG. 10).

DETD . . . induce neutralizing antibodies may be related to the fact that vP555 produces an extracellular particulate form of the structural proteins **E** and M. This SHA-like particle probably represents an empty JEV envelope that contains **E** and M folded and assembled into a configuration very similar to that found in the infectious JEV particle.

Recombinants vP555. . . assembly of viral envelopes. Other investigators (see above) have not been able to detect the production of extracellular forms of **E** by cells expressing all three structural proteins (C, **prM**, and **E**) in the presence or absence of NS1 and NS2A. The inability of their recombinant viruses to produce particles similar to. . .

DETD . . . were obtained from GIBCO/BRL, Gaithersburg, MD, New England Biolabs, Beverly, MA; and Boehringer Mannheim Biochemicals, Indianapolis, IN. Klenow fragment of **E**. coli polymerase was obtained from Boehringer Mannheim Biochemicals. BAL-31 exonuclease and phage T4 DNA ligase were obtained from New England. . .

DETD . . . NcoI site (pos. 172,253) were removed by digestion of pSD419 with NcoI/SmaI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation generating plasmid pSD476. A vaccinia right flanking arm was obtained by digestion of pSD422 with HpaI. . . ID NO:6/SEQ ID NO:7) ##STR3## generating pSD479. pSD479 contains an initiation codon (underlined) followed by a BamHI site. To place **E**. coli Beta-galactosidase in the B13-B14 (u) deletion locus under the control of the u promoter, a 3.2 kb BamHI fragment. . .

DETD . . . at the pUC/vaccinia junction was destroyed by digestion of pSD478 with EcoRI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation, generating plasmid pSD478E$^-$. pSD478E$^-$ was digested with BamHI and HpaI and ligated with annealed synthetic oligonucleotides. . .

DETD . . . XbaI within vaccinia sequences (pos. 137,079) and with HindIII at the pUC/vaccinia junction, then blunt ended with Klenow fragment of **E**. coli polymerase and ligated, resulting in plasmid pSD483. To remove unwanted vaccinia DNA sequences to the right of the A26L. . . digestion with BglII (pos. 140,136) and with EcoRI at the pUC/vaccinia junction, followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation. The resulting plasmid was designated pSD489. The 1.8 kb ClaI (pos. 137,198)/EcoRV (pos. 139,048) fragment from. . .

DETD A 3.3 kb BglII cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD A 3.2 kb BglII/BamHI (partial) cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . were removed from the pUC/vaccinia junction by digestion of pSD466 with EcoRI/BamHI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation. Recombination between vP708 and pSD467 resulted in recombinant: vaccinia deletion mutant, vP723, which was isolated as. . .

DETD To provide a substrate for the deletion of the [C7L-K1L] gene cluster from vaccinia, **E**. coli Beta-galactosidase was first inserted into the vaccinia M2L deletion locus (Guo et al., 1990) as follows. To eliminate the. . . unique BglII site inserted into the M2L deletion locus as indicated above. A 3.2 kb BamHI (partial)/BglII cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the 11 kDa promoter (Bertholet et al., 1985) was. . .

DETD . . . deleted for vaccinia genes [C7L-K1L] was assembled in PUCS cut with SmaI, HindIII and blunt ended with Klenow fragment of **E**. coli polymerase. The left flanking arm consisting of vaccinia HindIII C sequences was obtained by digestion of pSD420 with XbaI (pos. 18,628) followed by blunt ending with Klenow fragment of **E**. coli polymerase and digestion with BglII (pos. 19,706). The right flanking arm consisting of vaccinia HindIII K sequences was obtained. . .

DETD . . . coding sequences, pSD518 was digested with BamHI (pos. 65,381) and HpaI (pos. 67,001) and blunt ended using Klenow fragment of **E**. coli polymerase. This 4.8 kb vector fragment was ligated with a 3.2 kb SmaI cassette containing the **E**. coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . mutagenized expression cassette contained within pRW837 was derived by digestion with HindIII and EcoRI, blunt-ended using the Klenow fragment of **E**. coli DNA polymerase in the presence of 2mM dNTPs, and inserted into the SmaI site of pSD513 to yield pRW843.. . .

DETD . . . into pRW843 (containing the measles HA gene). Plasmid pRW843 was first digested with NotI and blunt-ended with Klenow fragment of **E**. coli DNA polymerase in the presence of 2mM dNTPs. The resulting plasmid, pRW857, therefore contains the measles virus F and. . .

DETD . . . of JEV. First strand cDNA synthesis was primed from a synthetic oligonucleotide complementary to bases 986 to 1005 of the **E** coding region of JEV (FIG. 17A and B) (SEQ ID NO:52). The double-stranded cDNA was ligated to synthetic oligonucleotides containing. . . Biolabs, Beverly, MA), inserted into phosphatase treated EcoRI-cleaved pBR322 (New England Biolabs), and the resulting DNA was used to transform **E**. coli strain DH5 cells (GIBCO/BRL). Plasmids were analyzed by restriction enzyme digestion and a plasmid (pC20) containing cDNA corresponding to

81 nucleotides of non-coding RNA and the C and **prM** coding regions was identified. pC20 was digested at the linker sites with EcoRI and at an internal DraI site situated 28 bp 5' of the ATG initiation codon and the resulting fragment containing the C and **prM** coding regions was inserted into SmaI-EcoRI digested pUC18, creating plasmid, pDr20. The sequence of the C coding region of pC20, combined with an updated sequence of the **prM**, **E**, NS1, NS2A, and NS2B coding regions of the Nakayama strain of JEV is presented in FIG. 17A and B (SEQ. . .

DETD . . . the XhoI and AccI fragment of JEV2 (FIG. 1) containing the plasmid origin and JEV cDNA encoding the carboxy-terminal 40% **prM** and amino-terminal two thirds of **E** (nucleotides 603 to 2124), generating plasmid JEV20 containing JE sequences from the ATG of C through the SacI site (nucleotide 2124) found in the last third of **E**.

DETD . . . 1) in which TTTTTGT nucleotides 1304 to 1310 were changed to TCTTTGT), containing JE sequences from the last third of **E** through the first two amino acids of NS2B (nucleotides 2124 to 4126), the plasmid origin and vaccinia sequences, was ligated. . .

DETD . . . end] generated plasmid JEV25 which contains JE cDNA extending from the SacI site (nucleotide 2124) in the last third of **E** through the carboxy-terminus of **E**. The SacI-EagI fragment from JEV25 was ligated to the SacI-EagI fragment of JEV8 (containing JE cDNA encoding 15 aa C, **prM** and amino-terminal two thirds of **E** nucleotides 337 to 2124, the plasmid origin and vaccinia sequences) yielding plasmid JEV26. A unique SmaI site preceding the ATG. . .

DETD . . . or HpaI-HindIII fragment from JEV7 (FIG. 2) yielded JEV29 [containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A (nucleotides 2293 to 4125)] and JEV30 [containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A, NS2B (nucleotides 2293 to 4512)].

DETD Recombinant vP825 encoded the capsid protein C, structural protein precursor **prM**, the structural glycoprotein **E**, the nonstructural glycoprotein NS1, and the nonstructural protein NS2A (McAda et al., 1987). Recombinant vP829 encoded the putative 15 aa signal sequence preceding the amino-terminus of **prM**, as well as **prM**, and **E** (McAda et al., 1987). Recombinant vP857 contained a cDNA encoding the 30 aa hydrophobic carboxy-terminus of **E**, followed by NS1 and NS2A. Recombinant vP864 contained a cDNA encoding the same proteins as vP857 with the addition of NS2B. In recombinants vP825 and vP829 a potential vaccinia virus early transcription termination signal in **E** (TTTTTGT; nucleotides 1399-1405) was modified to TCTTTGT without altering the aa sequence. This change was made in an attempt to increase the level of expression of **E** since this sequence has been shown to increase transcription termination in in vitro transcription assays (Yuen et al., 1987).

DETD **E** and **prM** Were Properly Processed When Expressed by Recombinant Vaccinia Viruses

DETD Pulse-chase experiments demonstrate that proteins identical in size to **E** were synthesized in cells infected with all recombinant vaccinia viruses containing the **E** gene (Table 3). In the case of cells infected with JEV, vP555 and vP829, an **E** protein that migrated slower in SDS-PAGE was also detected in the culture fluid harvested from the infected cells (Table 3). This extracellular form of **E** produced by JEV- and vP555-infected cells contained mature N-linked glycans (Mason, 1989; Mason et al., 1991), as confirmed for the extracellular forms of **E** produced by vP829-infected cells. Interestingly, vP825, which contained the C coding region in addition to **prM** and **E** specified the synthesis of **E** in a form that is not released into the extracellular fluid (Table 3). Immunoprecipitations prepared from radiolabeled vaccinia-infected cells using a MAb specific for M (and **prM**) revealed that **prM** was synthesized in cells infected with vP555, vP825, and vP829, and M was detected in the culture fluid of cells. . .

DETD . . . fluids (Table 3). This result indicated that vP829 infected cells produced extracellular particles similar to the empty viral envelopes containing **E** and M which are observed in the culture fluids harvested from vP555 infected cells (FIG. 9).

DETD Pre-challenge sera pooled from selected animals in each group were tested for their ability to immunoprecipitate radiolabeled **E** and NS1. The results of these studies (Table 3) demonstrated that: (1) the following order of immune response to **E** vP829>vP555>vP825, (2) all viruses encoding NS1 and NS2A induced antibodies to NS1, and (3) all immune responses were increased by. . . sera collected from these animals (Table 4) confirmed the results of the immunoprecipitation analyses, showing that the immune response to **E** as demonstrated by RIP correlated well with these other serological tests (Table 4).

DETD TABLE 3

Characterization of proteins expressed by vaccinia recombinants and their immune responses

	vP555	vP829	vP825	vP857	vP864

Proteins expressed					
Intracellular	**prM,E** NS1	**prM,E**	**prM,E** NS1	NS1	NS1
secreted	M,**E**,NS1	M,**E**	NS1	NS1	NS1
Particle formation	+	+	-	-	-
Immune response					
single	**E**	**E**	NS1	NS1	NS1
double	**E**,NS1	**E**	**E**,NS1	NS1	NS1

single = single inoculation with 10^7 pfu vaccinia recombinants (ip)
double = two inoculations with 10^7 pfu vaccinia. . .

DETD . . . isolated and ligated to a SacI (JEV nucleotide 2125) to EagI fragment of JEV25 (containing the remaining two thirds of **E**, translation stop and T5NT) generating JEV36. JEV36 was transfected into vP866 (NYVAC) infected cells to generate the vaccinia recombinant vP923. . .

DETD Plasmid YFO containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) was derived by cloning an AvaI to NsiI fragment of YF cDNA (nucleotides 537-1658). . . and KpnI digested IBI25 (International Biotechnologies, Inc., New Haven, CT). Plasmid YF1 containing YF cDNA encoding C and amino-terminal 20% **prM** (nucleotides 119-536) was derived by cloning a RsaI to AvaI fragment of YF cDNA (nucleotides 166-536) and annealed oligos SP46. . . and YF nucleotides 119-165) into AvaI and HindIII digested IBI25. Plasmid YF3 containing YF cDNA encoding the carboxy-terminal 60% of **E** and amino-terminal 25% of NS1 was generated by cloning an ApaI to BamHI fragment of YF CDNA (nucleotides 1604-2725) into. . . cDNA (nucleotides 4339-4940) into SacI and XbaI digested IBI25. Plasmid YF13 containing YF cDNA encoding the carboxy-terminal 25% of C, **prM** and amino-terminal 40% of **E** was derived by cloning a BalI to AnaI fragment of YF cDNA (nucleotides 384-1603) into ApaI and SmaI digested IBI25.

DETD . . . gene in YF1 (TTTTTCT nucleotides 263-269 and TTTTTGT nucleotides 269-275) to (SEQ ID NO:35) TTCTTCTTCTTGT creating plasmid YF1B, in the **E** gene in YF3 (nucleotides 1886-1893 TTTTTTGT to TTCTTTGT 189 aa from the carboxy-terminus and nucleotides 2429-2435 TTTTTGT to TTCTTGT 8. . . YF3C (nucleotides 1965-2725) was exchanged for the corresponding fragment of YF3B generating YF4 containing YF CDNA encoding the carboxy-terminal 60% **E** and amino-terminal 25% NS1 (nucleotides 1604-2725) with both mutagenized transcription termination signals. An ApaI to BamHI fragment from YF4 (nucleotides 1604-2725) was substituted for the equivalent region in YFO creating plasmid YF6 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with both mutagenized transcription termination signals. Plasmid YF6 was digested with EcoRV within the. . .

DETD . . . mutagenesis described above was used to insert XhoI and ClaI sites preceding the ATG 17 aa from the carboxy-terminus of **E** (nucleotides 2402-2404) in plasmid YF3C creating YF5, to insert XhoI and ClaI sites preceding the ATG 19 aa from the carboxy-terminus of **prM** (nucleotides 917-919) in plasmid YF13 creating YF14, to insert an XhoI site preceding the ATG 23 aa from the carboxy-terminus of **E** (nucleotides 2384-2386) in plasmid YF3C creating plasmid YF25, and to insert an XhoI site and ATG (nucleotide 419) in plasmid. . .

DETD . . . YF5 (nucleotides 1604-2725) was exchanged for the corresponding region of YFO creating YF7 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at 2402 (17 aa from the carboxy-terminus of **E**) and a mutagenized transcription termination signal at 2429-2435 (8 aa from the carboxy-terminus of **E**). The ApaI to BamHI fragment from YF25 (nucleotides 1604-2725) was exchanged for the corresponding region of YFO generating YF26 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with an XhoI site at nucleotide 2384 (23 aa from the carboxy-terminus of **E**) and mutagenized transcription termination signal at 2428-2435 (8 aa from the carboxy-terminus of **E**).

DETD . . . YF14 (nucleotides 537-1603) was substituted for the corresponding region in YF6 generating YF15 containing YF cDNA encoding the carboxy-terminal 80% **prM**, **E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at nucleotide 917 (19 aa from the carboxy-terminus of **prM**) and two mutagenized transcription termination signals. YF6 was digested within IBI25 with EcoRV and within YF at nucleotide 537 with. . .

DETD . . . from YF12 encoding carboxy-terminal 20% NS1, NS2A and NS2B (nucleotides 3267-4569), XhoI to KpnI fragment from YF1S encoding 19 aa **prM**, **E** and amino-terminal 80% NS1 (nucleotides 917-3266) and XhoI-SmaI digested pHES4 were ligated generating YF23. An XhoI to BamHI

fragment from YF26 encoding 23 aa **E**, amino-terminal 25% NS1 (nucleotides 2384-2725) was ligated to an XhoI to BamHI fragment from YF23 (containing the carboxy-terminal 75% NS1,. . .

DETD XhoI-SmaI digested pHES4 was ligated to a purified XhoI to XpnI fragment from YF7 encoding 17 aa **E** and amino-terminal 80% NS1 (nucleotides 2402-3266) plus a KpnI to SmaI fragment from YF10 encoding the carboxy-terminal 20% NS1 and NS2A (nucleotides 3267-4180) creating YF18. An XhoI to BamHI fragment from YF2 encoding C, **prM**, **E** and amino-terminal 25% NS1 (nucleotides 119-2725) was ligated to a XhoI to BamHI fragment of YF18 (containing the carboxy-terminal 75%. . . the origin of replication and vaccinia sequences) generating YF20. A XhoI to BamHI fragment from YF46 encoding 21 aa C, **prM**, **E** and amino-terminal 25% NS1 (nucleotides 419-2725) was ligated to the XhoI to BamHI fragment from YF18 generating YF47. Oligonucleotide SP46. . .

DETD Radiolabeled cell lysates and culture fluids were harvested and the viral proteins were immunoprecipitated with monoclonal antibodies to YF **E** and NS1 and separated in SDS-containing polyacrylamide gels exactly as described by Mason (1989).

DETD . . . NS1 and NS2A (Rice et al., 1985). Recombinant vP729 encoded the putative 19-aa signal sequence preceding the N terminus of **E**, **E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP764 encoded C, **prM**, **E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP766 encoded C, **prM**, **E**, NS1 and NS2A (Rice et al., 1985). Recombinant vP869 encoded the putative 21-aa signal sequence preceding the N terminus of the structural protein precursor **prM**, **prM E**, NS1 and NS2A (Rice et al., 1985).

DETD **E** Protein Expression By Recombinant Vaccinia Virus

DETD Pulse-chase experiments in HeLa cells demonstrated that a protein identical in size to YF17D **E** was synthesized in cells infected with vP869 and secreted into the culture fluid (Table 7). Under the same conditions of labeling, no intracellular or extracellular **E** was detected in cultures infected with vP766, vP729 or the control vaccinia virus vP457 (Table 7).

DETD Continuous label experiments in Vero cells demonstrated that a protein identical in size to the **E** protein expressed by vP869 was expressed in cultures infected with vP766 and vP729 (Table 7). These results suggest that the **E** protein produced by vP869 infected cells is present in a form in which it is more stable than the **E** protein expressed by vP766 or vP729. YF17D has previously been shown to produce a more labile **E** protein than other YF isolates (Cane et al. 1989).

DETD . . . immunization with YF17D. Pre-challenge sera pooled from selected animals in each group were tested for their ability to immunoprecipitate radiolabeled **E** and NS1 proteins and for the presence of Neut and HAI antibodies. As shown in Table 9 only vP869 and YF17D immunized mice responded to **E** protein, the response was increased by a second inoculation. Mice immunized twice with vP729, vP725 or vP766 produced antibody to. . .

DETD TABLE 7

Characterization of proteins expressed by vaccinia recombinants and YF17D

	17D	vP869	vP729	vP725	vP766	vP457
YF Proteins Expressed Intracellular	**E**,NS1	**E**	**E**,NS1	NS1	**E**,NS1	NONE
Secreted	**E**,NS1	**E**	NS1	NS1	NONE	NONE
Extracellular	YES	YES	NO	NO	NO	NO
HA Activity						

DETD TABLE 9

Pre-challenge Radioimmunoprecipitation

Immunizing Virus	One Inoculation		Two Inoculations	
	Anti-**E**	Anti-NS1	Anti-**E**	Anti-NS1
vP457	-	-	-	-
vP725				+
vP729				+
vP766				+
vP869	+	-	++	-
17D	+	-	++	-

DETD A XhoI to SmaI fragment from YF47 (nucleotides 419-4180) containing YF CDNA encoding 21 amino acids C, **prM**, **E**, NS1, NS2A (with a base

missing in NS1 nucleotide 2962) was ligated to XhoI-SmaI digested SPHA-H6 (HA region donor plasmid). . . (nucleotide 3262) and a 6700 bp fragment isolated (containing the plasmid origin of replication, vaccinia sequences, 21 amino acids C, **prM**, **E**, amino-terminal 3.5% NS1, carboxy-terminal 23% NS1, NS2A) and ligated to a SacI-Asp718 fragment from YF18 (containing the remainder of NS1. . . site in YF51 were removed using oligonucleotide-directed double-strand break mutagenesis (Mandecki, 1986) creating YF50 encoding YF 21 amino acids C, **prM**, **E**, NS1, NS2A in the HA locus donor plasmid. YF50 was transfected into vP866 (NYVAC) infected cells generating the recombinant vP984. . .

DETD . . . double-strand break mutagenesis creating YF49. Oligonucleotide-directed mutagenesis (Kunkel, 1985) was used to insert a SmaI site at the carboxy-terminus of **E** (nucleotide 2452) in YF4 creating YF16. ApaI-SmaI fragment of YF49 (containing the plasmid origin of replication, vaccinia sequences and YF CDNA encoding 21 amino acids C, **prM**, and amino-terminal 43% **E**) was ligated to an ApaI-SmaI fragment from YF16 (nucleotides 1604-2452 containing the carboxy-terminal 57% **E**) generating YF53 containing 21 amino acids C, **prM**, **E** in the HA locus donor plasmid. YF53 was transfected into vP866 (NYVAC) infected cells generating the recombinant vP1003 (FIG. 19).. . .

DETD . . . digested pUC8. An EcoRI-HindIII fragment from DEN1 (nucleotides 2559-3745) and SacI-EcoRI fragment of DEN CDNA encoding the carboxy-terminal 36% of **E** and amino-terminal 16% NS1 (nucleotides 1447-2559, Mason et al., 1987B) were ligated to HindIII-SacI digested IBI24 (International Biotechnologies, Inc., New Haven, Conn.) generating DEN3 encoding the carboxy-terminal 64% **E** through amino-terminal 45% NS2A with a base missing in NS1 (nucleotide 2467).

DETD . . . an AvaI-SacI fragment of DEN cDNA (nucleotides 424-1447 Mason et al., 1987B) generating DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E**.

DETD Plasmid DEN6 containing DEN cDNA encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579 with nucleotide 2467 present Mason et al., 1987B) was derived by cloning a SacI-XhoI. . . Biotechnologies, Inc., New Haven, Conn.). Plasmid DEN15 containing DEN cDNA encoding 51 bases of the DEN 5' untranslated region, C, **prM** and amino-terminal 36% **E** was derived by cloning a HindIII-SacI fragment of DEN cDNA (nucleotides 20-1447, Mason et al., 1987B) into HindIII-SacI digested IBI25.. . .

DETD . . . mutagenesis (Kunkel, 1985) was used to change potential vaccinia virus early transcription termination signals (Yuen et al., 1987) in the **prM** gene in DEN4 29 aa from the carboxy--terminus (nucleotides 822-828 TTTTTCT to TATTTCT) and 13 aa from the carboxy-terminus (nucleotides. . .

DETD . . . (nucleotide 4102) in plasmid DEN23 creating DEN24, to insert a SmaI site and ATG 15 aa from the carboxy-terminus of **E** in DEN7 (nucleotide 2348) creating DEN10, to insert an EagI and HindIII site at the carboxy-terminus of NS2B (nucleotide 4492). . .

DETD . . . DEN7 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN19 containing DEN CDNA encoding the carboxy-terminal 64% **E** and amino-terminal 45% NS2A (nucleotides 1447-3745) with nucleotide 2467 present and the modified transcription termination signal (nucleotides 2448-2454). A XhoI-XbaI. . .

DETD . . . of the H6 promoter and DEN nucleotides 68-494) was ligated to a HindIII-PstI fragment from DEN47 (encoding the carboxy-terminal 83% **prM** and amino-terminal 36% of **E** nucleotides 494-1447 and plasmid origin of replication) generating DEN17 encoding C, **prM** and amino-terminal 36% **E** with part of the H6 promoter and EcoRV site preceding the amino-terminus of C. A HindIII-BlII fragment from DEN17 encoding the carboxy-terminal 13 aa C, **prM** and amino--terminal 36% **E** (nucleotides 370-1447) was ligated to annealed oligonucleotides SP111 and SP112 (containing a disabled HindIII sticky end, EcoRV site to -1. . . a BglII sticky end) creating DEN33 encoding the EcoRV site to -1 of the H6 promoter, carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**.

DETD . . . digested pTP15 (Mason et al., 1991) was ligated to a SmaI-SacI fragment from DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E** (nucleotides 377-1447) and SacI-EagI fragment from DEN3 encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A generating DENL. The SacI-XhoI fragment from DEN7 encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579) was ligated to a BstEII-SacI fragment from DEN47 (encoding the carboxy-terminal 55% **prM** and amino-terminal 36% **E** (nucleotides 631-1447) and a BstEII-XhoI fragment from DENL (containing the carboxy-terminal 11 aa C, amino-terminal 45% **prM**, carboxy-terminal 82% NS1, carboxy-terminal 45% NS2A, origin of replication and vaccinia sequences) generating DEN8. A unique SmaI site (located between. . .

DETD An EcoRV-SacI fragment from DEN17 (positions -21 to -1 H6 promoter DEN nucleotides 68-1447) encoding C, **prM** and amino-terminal terminal 36% **E**) was ligated to an EcoRV-SacI fragment of DEN8VC (containing vaccinia sequences, H6 promoter from -21 to -124, origin of replication and amino-terminal 64% **E**, NS1, amino-terminal terminal 45% NS2A nucleotides 1447-3745) generating DEN18. A XhoI-EagI fragment from DEN25 encoding the carboxy-terminal 82% NS1 and. . . (nucleotides 2579-4102) was ligated to an XhoI-EagI fragment of DEN18 (containing the origin of replication, vaccinia sequences and DEN C **prM**, **E** and amino-terminal 18% NS1 nucleotides 68-2579) generating DEN26. An EcoRV-SacI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN nucleotides 377-1447 encoding 11aaC, **prM** and amino-terminal 36% **E**) was ligated to an EcoRV-SacI fragment of DEN26 (containing the origin of replication, vaccinia sequences and DEN region encoding the carboxy-terminal 64% **E**, NS1 and NS2A with a base missing in NS1 at nucleotide 2894) generating DEN32. DEN32 was transfected into vP410 infected. . .

DETD . . . DEN10 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN11 containing DEN CDNA encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A with a SmaI site and ATG 15 aa from the carboxy-terminus of **E**. A SmaI-EagI fragment from DEN11 (encoding the carboxy-terminal 15 aa **E**, NS1 and amino-terminal 45% NS2A nucleotides 2348-3745) was ligated to SmaI-EagI digested pTP15 generating DEN12.

DETD An EcoRV-XhoI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN nucleotides 377-2579 encoding the carboxy-terminal 11 aa C, **prM** **E**, amino-terminal 18% NS1) was ligated to an EcoRV-XhoI fragment from DEN31 (containing the origin of replication, vaccinia sequences and DEN. . . An EcoRV-SacI fragment from DEN33 (positions -21 to -1 H6 promoter DEN nucleotides 350-1447 encoding the carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**) and a SacI-XhoI fragment from DEN32 (encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 nucleotides 1447-2579) were ligated to the EcoRV-SacI fragment from DEN31 described above generating DEN34. DEN34 was. . .

DETD Construction of Vaccinia Insertion Vector Containing DEN Type 1 20 aaC, **prM**, **E**

DETD A 338bp fragment encoding the carboxy-terminal 23% **E** (nucleotides 2055-2392, Mason et al., 1987b) TGA stop codon T5NT vaccinia early transcription termination signal (Yuen et al., 1987) and. . . and cloned into HindIII/BamHI digested IBI25 generating DEN36. DEN34 was digested with EcoRV (within the H6 promoter) and HindIII within **E** (DEN nucleotide 2061; Mason et al., 1987b) and a 1733 bp fragment (containing EcoRV to -1 H6 promoter, 20 aaC, **prM** and amino-terminal 77% **E**) was isolated. DEN36 was digested with HindIII and EclXI and a 331 bp fragment isolated (containing DEN nucleotides 2062-2392 TGA. . . generating plasmid DEN38. Plasmid DEN38 can be transfected into vaccinia infected cells to generate a recombinant encoding DEN 20 aaC, **prM** and **E**.

DETD This example describes the development of canarypox recombinant vCP107 encoding JEV 15aaC, **prM**, **E**, NS1, NS2A and a canarypox donor plasmid (JEVCPC5) encoding 15aaC, **prM**, **E**.

DETD Construction of Insertion Vector Containing JEV 15aaC, **prM**, **E**, NS1, NS2A

DETD Construction of pRW838 is illustrated below (FIG. 23). Oligonucleotides A through **E**, which overlap the translation initiation codon of the H6 promoter with the ATG of rabies G, were cloned into pUC9 as pRW737. Oligonucleotides A through **E** contain the H6 promoter, starting at NruI, through the HindIII site of rabies G followed by BglII. Sequences of oligonucleoties A through **E** are: ##STR16## The diagram of annealed oligonucleotides A through **E** is as follows: ##STR17##

DETD Oligonucleotides A through **E** were kinased, annealed (95° C. for 5 minutes, then cooled to room temperature), and inserted between the PvuII sites of. . .

DETD . . . promoter, plasmid origin of replication and C5 flanking arms isolated. Plasmid JEVL14VC containing JEV cDNA encoding 15 amino acids C, **prM**, **E**, NS1, NS2A in a vaccinia virus donor plasmid (FIG. 1) (nucleotides 337-4125, FIG. 17A and B) (SEQ ID NO:52) was. . . JEVCP1 was transfected into ALVAC infected primary CEF cells to generate the canarypox recombinant vCP107 encoding 15 amino acids C, **prM**, **E**, NS1, NS2A (FIG. 18).

DETD Construction of C5 Insertion Vector Containing JEV 15aac, **prM**, **E**

DETD . . . annealed oligonucleotides SP131 (SEQ ID NO:75) and SP132 (SEQ ID NO:76) (containing a SphI sticky end, T nucleotide completing the **E** coding region, translation stop, a vaccinia early transcription termination signal (AT5AT; Yuen and Moss, 1987), a second translation stop, and XbaI sticky end) generating plasmid JEVCP5 which encodes 15 amino acids C, **prM** and **E** under the control of the H6 promoter between C5 flanking arms. JEVCP5 can be transfected in ALVAC or ALVAC recombinant infected cells to generate a recombinant encoding JEV 15 aa C, **prM** and **E**. ##STR19## Example 15--CONSTRUCTION OF ALVAC RECOMBINANT EXPRESSING YFV PROTEINS Construction of Canarypox Insertion

Vector

DETD . . . and SmaI and ligated to a 3772 bp XhoI-SmaI fragment from YF51 (nucleotides 419-4180 encoding YF 21 amino acids C, **prM**, **E**, NS1, NS2A) generating YF52. The 6 bp corresponding to the unique XhoI site in UP52 were removed using oligonucleotide-directed double-strand. . . YFCP3. YFCP3 was transfected into ALVAC infected primary CEF cells to generate the canarypox recombinant vCPI27 encoding 21 aa C, **prM**, **E**, NS1, NS2A (FIG. 19).

DETD Construction of C3 Insertion Vector Containing YFV 21 aa C, **prM**, **E**

DETD . . . 8344 bp fragment isolated (containing the plasmid origin of replication, canarypox DNA and YF cDNA encoding 21 amino acids C, **prM**, and amino-terminal 57% **E**) and ligated to a ApaI to SmaI fragment from YF16 (nucleotides 1604-2452 containing the carboxy-terminal 43% **E**) generating YF54. The 6 bp corresponding to the unique XhoI site in YF54 were removed as described above creating YFCP4 containing YF cDNA encoding 21 amino acids C, **prM**, and **E**. YFCP4 can be transfected into ALVAC or ALVAC recombinant infected cells to generate a recombinant encoding YFV 21 aa C, **prM**, **E**.

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CLM What is claimed is:
1. A recombinant poxvirus comprising DNA coding for at least one flavivirus structural protein, wherein the flavivirus is **Yellow Fever virus** or Dengue virus and the poxvirus is selected from the group consisting of: an avipox virus, a vaccinia virus wherein. . . . . recombinant poxvirus of claim 1 wherein the DNA encodes protein M or a precursor to protein M, and flavivirus proteins **E**, NS1 and NS2A.

7. The recombinant poxvirus of claim 1 wherein the flavivirus is **Yellow Fever virus**.

=> d l6,cbib,7

L6 ANSWER 7 OF 7 USPATFULL on STN
1998:44886 Flavivirus recombinant poxvirus immunological composition.
Paoletti, Enzo, Delmar, NY, United States
Pincus, Steven Elliot, East Greenbush, NY, United States
Virogenetics Corporation, Troy, NY, United States (U.S. corporation)
US 5744141 19980428
APPLICATION: US 1995-484304 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l6,cbib,kwic,7

L6 ANSWER 7 OF 7 USPATFULL on STN
1998:44886 Flavivirus recombinant poxvirus immunological composition.
Paoletti, Enzo, Delmar, NY, United States
Pincus, Steven Elliot, East Greenbush, NY, United States
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US 5744141 19980428
APPLICATION: US 1995-484304 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB . . . poxvirus, such as vaccinia virus, fowlpox virus and canarypox virus, containing foreign DNA from flavivirus, such as Japanese encephalitis virus, **yellow fever virus** and Dengue virus. In a preferred embodiment, the recombinant poxvirus generates an extracellular particle containing flavivirus **E** and M proteins capable of inducing neutralizing antibodies, hemagglutination-inhibiting antibodies and protective immunity against flavivirus infection. What is also described. . .
SUMM . . . sequence to be inserted into the virus, particularly an open reading frame from a non-pox source, is placed into an **E**. coli plasmid construct into which DNA homologous to a section of DNA of the poxvirus has been inserted.
SUMM . . . flanking a region of pox DNA containing a nonessential locus. The resulting plasmid construct is then amplified by growth within **E**. coli bacteria (Clewell, 1972) and isolated (Clewell et al., 1969; Maniatis et al., 1986).
SUMM Second, the isolated plasmid containing the DNA gene sequence to be inserted is transfected into a cell culture, **e**.g. chick embryo fibroblasts, along with the poxvirus. Recombination between homologous pox DNA in the plasmid and the viral genome respectively. . .
SUMM . . . the nonstructural glycoprotein NS1 and the remaining nonstructural proteins (Rice et al., 1985). The flavivirus virion contains an envelope glycoprotein, **E**, a membrane protein, M, and a capsid protein, C. In the case of Japanese encephalitis virus (JEV), virion preparations usually contain a small amount of the glycoprotein precursor to the membrane protein, **prM** (Mason et al., 1987a). Within JEV-infected cells, on the other hand, the M protein is present almost exclusively as the higher molecular weight **prM** protein (Mason et al., 1987a; Shapiro et al., 1972).
SUMM . . . that have examined the protective effect of passively administered monoclonal antibodies (MAbs) specific for each of the three flavivirus glycoproteins (**prM**, **E**, NS1) have demonstrated that immunity to each of these antigens results in partial or complete protection from lethal viral challenge. Monoclonal antibodies to **E** can provide protection from infection by Japanese encephalitis virus (JEV) (Kimura-Kuroda et al., 1988; Mason et al., 1989), dengue type 2 virus (Kaufman et al., 1987) and **yellow fever virus** (YF) (Gould et al., 1986). In most cases, passive protection has been correlated with the ability of these **E** MAbs to neutralize the virus in vitro. Recently, Kaufman et al. (1989) have demonstrated that passive protection can also be produced with **prM** MAbs that exhibit weak or undetectable neutralizing activity in vitro. The ability of structural protein

specific MAbs to protect animals. . . attenuate viral infection by blocking virus binding to target cells. Passive protection experiments using MAbs to the NS1 protein of **yellow fever virus** (Schlesinger et al., 1985; Gould et al., 1986) and dengue type 2 virus (Henchal et al., 1988) have demonstrated that. . .

SUMM . . . of NS1 immunity to protect the host from infection comes from direct immunization experiments in which NS1 purified from either **yellow fever virus**-infected cells (Schlesinger et al., 1985, 1986) or dengue type 2 virus-infected cells (Schlesinger et al., 1987) induced protective immunity from. . .

SUMM . . . NS1-based vaccines, dimerization of NS1 (Winkler et al., 1988) may be required to elicit the maximum protective response. For the **E** protein, correct: folding is probably required for eliciting a protective immune response since **E** protein antigens produced in **E**. coli (Mason et al., 1989) and the authentic **E** protein prepared under denaturing conditions (Wengler et al., 1989b) failed to induce neutralizing antibodies. Correct folding of the **E** protein may require the coordinated synthesis of the **prM** protein, since these proteins are found in heterodimers in the cell-associated forms of West Nile virus (Wengler et al., 1989a). The proper folding of **E** and the assembly of **E** and **prM** into viral particles may require the coordinated synthesis of the NS1 protein, which is coretained in an early compartment of the secretory apparatus along with immature forms of **E** in JEV-infected cells (Mason, 1989).

SUMM . . . a vaccinia recombinant containing the region of JEV encoding 65 out of the 127 amino acids of C, all of **prM**, all of **E**, and 59 out of the 352 amino acids of NS1. Haishi et al. (1989) reported a vaccinia recombinant containing Japanese encephalitis sequences encoding 17 out of the 167 amino acids of **prM**, all of **E** and 57 out of the 352 amino acids of NS1.

SUMM Deubel et al. (1988) reported a vaccinia recombinant containing the dengue-2 coding sequences for all of C, all of **prM**, all of **E** and 16 out of the 352 amino acids of NS1.

SUMM Zhao et al. (1987) reported a vaccinia recombinant containing the dengue-4 coding sequences for all of C, all of **prM**, all of **E**, all of NS1, and all of NS2A. Bray et al. (1989) reported a series of vaccinia recombinants containing the dengue-4 coding sequences for (i) all of C, all of **prM** and 416 out of the 454 amino acids of **E**, (ii) 15 out of the 167 amino acids of **prM** and 416 out of the 454 amino acids of **E**, (iii) 18 amino acids of influenza A virus hemagglutinin and 416 out of the 454 amino acids of **E**, and (iv) 71 amino acids of respiratory syncytial virus G glycoprotein and 416 out of the 454 amino acids of **E**.

SUMM Despite these attempts to produce recombinant flavivirus vaccines, the proper expression of the JEV **E** protein by the vaccinia recombinants has not been satisfactorily obtained. Although Haishi et al. (1989) demonstrated cytoplasmic expression of JEV **E** protein by their vaccinia recombinant, the distribution was different from that observed in JEV infected cells. Yasuda et al. (1990) detected expression of JEV **E** protein by their vaccinia recombinant on the cell surface. Recombinant viruses that express the **prM** and **E** protein protected mice from approximately 10 LD_{50} of challenge virus. Yasuda et al. (1990) elicited anti-JEV immune responses as well as protection but reactivity to a panel of **E** specific monoclonal antibodies exhibited differences from the reactivity observed in JEV infected cells.

SUMM . . . the viral ORF extending from C to NS2A under the control of the P7.5 early-late promoter produced intracellular forms of **prM**, **E**, and NS1 but failed to induce the synthesis of extracellular forms of any of the structural proteins, even though a. . . (Bray et al., 1989; Zhao et al., 1987). Additional recombinant viruses that contained several forms of the dengue type 4 **E** gene with or without other structural protein genes have also been examined (Bray et al., 1989). Although several of these recombinant viruses were able to induce protection, they neither produced extracellular forms of **E** nor induced neutralizing antibodies. A dengue-vaccinia recombinant expressing a C-terminally truncated **E** protein gene induced the synthesis of an extracellular form of **E** and provided an increasing level of resistance to dengue virus encephalitis in inoculated mice (Men et al., 1991).

SUMM . . . protein capable of inducing protective immunity against flavivirus infection. In particular, the recombinant poxvirus generates an extracellular particle containing flavivirus **E** and M proteins capable of eliciting neutralizing antibodies and hemagglutination-inhibiting antibodies. The poxvirus is advantageously a vaccinia virus or an avipox virus, such as fowlpox virus or canarypox virus. The flavivirus is advantageously Japanese encephalitis virus, **yellow fever virus** and Dengue virus.

SUMM . . . In particular, the DNA contains Japanese encephalitis virus coding sequences that encode a precursor to structural protein M, structural protein **E**, and nonstructural proteins NS1 and NS2A. More in particular, the recombinant poxvirus contains therein DNA from

flavivirus in a nonessential region of the poxvirus genome for expressing a particle containing flavivirus structural protein **E** and structural protein M.

SUMM More in particular, the recombinant viruses express portions of the flavivirus ORF extending from **prM** to NS2B. Biochemical analysis of cells infected with the recombinant viruses showed that the recombinant viruses specify the production of properly processed forms of all three flavivirus glycoproteins--**prM**, **E**, and NS1. The recombinant viruses induced synthesis of extracellular particles that contained fully processed forms of the M and **E** proteins. Furthermore, the results of mouse immunization studies demonstrated that the induction of neutralizing antibodies and high levels of protection. . .

DRWD FIG. 7 shows a comparison by SDS-PAGE analysis of the cell lysate **E** proteins produced by JEV infection and infection with the recombinant vaccinia viruses vP650, vP555, vP658 and vP583;

DRWD FIG. 8 shows a comparison by SDS-PAGE analysis of the culture fluid **E** proteins produced by JEV infection and infection with the recombinant vaccinia viruses vP650, vP555, vP658 and vP583;

DRWD FIG. 9 shows a comparison by sucrose gradient analysis of the forms of the **E** protein found in the culture fluid harvested from JEV infected cells and cells infected with vaccinia recombinants vP555 and vP650;

DETD . . . and B (SEQ ID NO:52) which contains the sequence of the C coding region combined with an updated sequence of **prM**, **E**, NS1, NS2A and NS2B coding regions.

DETD . . . The resulting plasmid, pJEV1, contained the viral ORF extending from the SacI site (nucleotide 2125) in the last third of **E** through the BalI site (nucleotide 4125) two amino acid residues (aa) into the predicted N terminus of NS2B (FIG. 1).

DETD . . . containing a XhoI sticky end, a SmaI site, the last 15 aa of C, and first 9 aa of JEV **prM** with a sticky HindIII end) were ligated to a HindIII-SacI fragment of JEV CDNA (nucleotides 407-2124), and XhoI-SacI digested vector. . . the viral ORF extending between the methionine (Met) codon (nucleotides 337-339) occurring 15 aa preceding the predicted N terminus of **prM** and the SacI site (nucleotide 2124) found in the last third of **E** (FIG. 1).

DETD . . . pJEV5, contained the viral ORF extending between the Met codon (nucleotides 811-813) occurring 25 aa preceding the N terminus of **E** and the SacI site (nucleotide 2124) found in the last third of **E** (FIG. 1).

DETD . . . (Kunkel, 1985) was used to change a potential vaccinia virus early transcription termination signal (Yuen et al., 1987) in the **E** gene of pJEV2 (TTTTTGT; nucleotides 1304-1310) to TCTTTGT, creating plasmid pJEV22 (FIG. 2). The same change was performed on pJEV5. . .

DETD . . . resulting plasmid, pJEV7, contained the viral ORF extending between the SacI site (nucleotide 2125) found in the last third of **E** and the last codon of NS2B (nucleotide 4512) (FIG. 2). SmaI-EagI digested pTP15 was purified and ligated to the purified. . .

DETD Four different vaccinia virus recombinants were constructed that expressed portions of the JEV coding region extending from **prM** through NS2B. The JEV cDNA sequences contained in these recombinant viruses are shown in FIG. 4. In all four recombinant. . .

DETD Recombinant vP555 encodes the putative 15 aa signal sequence preceding the N terminus of the structural protein precursor **prM**, the structural glycoprotein **E**, the nonstructural glycoprotein NS1, and the nonstructural protein NS2A (McAda et al., 1987). Recombinant vP583 encodes the putative signal sequence preceding the N terminus of **E**, **E**, NS1, and NS2A (McAda et al., 1987). Recombinant vP650 contains a cDNA encoding the same proteins as vP555 with the. . . vP583 with the addition of NS2B. In recombinants vP650 and vP658, a potential vaccinia virus early transcription termination signal in **E** (TTTTTGT; nucleotides 1087-1094) was modified to TCTTTGT without altering the aa sequence. This change was made in an attempt to increase the level of expression of **E** and NS1, since this sequence has been shown to increase transcription termination in in vitro transcription assays (Yuen et al.,. . .

DETD . . . production by all four recombinants, suggesting that the potential vaccinia early transcriptional termination signal present near the end of the **E** coding region in vP555 and vP583 did not significantly reduce the amount of NS1 produced relative to vP650 or vP658. . .

DETD **E** and **prM** were Properly Processed when Expressed by Recombinant Vaccinia Viruses

DETD FIGS. 7 and 8 show a comparison of the **E** protein produced by JEV infection or infection with the recombinant vaccinia viruses. BHK cells were infected with JEV or recombinant. . .

DETD The data from the pulse-chase experiments depicted in FIGS. 7 and 8 demonstrate that proteins identical in size to **E** were synthesized in cells infected with all recombinant vaccinia viruses containing the **E** gene. However, the **E** protein was only released from cells infected

with vaccinia viruses that contained the region of the viral ORF encoding **prM**, **E**, NS1, and NS2A (vP555 and vP650; see FIGS. 4, 7 and 8). Endoglycosidase sensitivity (FIGS. 7 and 8) revealed that both the intracellular and extracellular forms of the **E** protein synthesized by cells infected with the vaccinia recombinants were glycosylated; the cell-associated forms of **E** were endo H sensitive, whereas the extracellular forms were resistant to endo H digestion.

DETD Immunoprecipitates prepared from radiolabeled vaccinia-infected cells using a MAb specific for M (and **prM**) revealed that **prM** was synthesized in cells infected with vP555 and vP650. Cells infected with either of these recombinant vaccinia viruses produced cellular forms of **prM** that were identical in size to the **prM** protein produced by JEV-infected cells, and a M protein of the correct size was detected in the culture fluid of. . .

DETD The extracellular fluid harvested from cells infected with vP555 and vP650 contained forms of **E** that migrated with a peak of hemagglutinating activity in sucrose density gradients. Interestingly, this hemagglutinin migrated similarly to the slowly. . .

DETD Recombinant vaccinia virus vP555 produced **E**- and M-containing extracellular particles that behaved like empty viral envelopes. The ability of this recombinant virus to induce the synthesis. . .

DETD . . . described herein contain portions of the JEV ORF that encode the precursor to the structural protein M, the structural protein **E**, and nonstructural proteins NS1, NS2A, and NS2B. The **E** and NS1 proteins produced by cells infected with these recombinant viruses underwent proteolytic cleavage and N-linked carbohydrate addition in a. . . proteins produced by cells infected with JEV. These data further demonstrate that the proteolytic cleavage and N-linked carbohydrate addition to **E** and NS1 do not require flavivirus nonstructural proteins located 3' to NS2A in the viral genome (Bray et al., 1989;. . .

DETD . . . the portion of the ORF inserted in the recombinant vaccinia viruses had a significant effect on the late-stage processing of **prM** and **E**, but not on the fate of NS1. All recombinant viruses that encoded NS1 produced mature extracellular forms of this protein,. . . from transfected cells (Fan et al., 1990). On the other hand, only two of the four recombinants that contained the **E** protein coding region produced extracellular forms of **E**. These two recombinants, vP555 and vP650, differed from the remaining recombinants in that they contained the **prM** coding region in addition to **E**, NS1, and NS2A. The findings that extracellular forms of **E** were produced only by viruses containing the coding regions for both **E** and **prM** and that the extracellular forms of **E** were associated with M suggest that the simultaneous synthesis of **prM** and **E** is a requirement for the formation of particles that are targeted for the extracellular fluid.

DETD . . . the co-migration of the rabbit immunoglobulin heavy chain with the radiolabeled viral antigens, and to permit clear separation of the **E** and the NS1' proteins. Neutralization tests were performed on heat-inactivated sera (20 min. at 56° C.) as described (Tesh et. . .

DETD . . . virus, two viruses (vP555 and vP658) were selected for in-depth challenge studies. vP555 induced the synthesis of extracellular forms of **E**, whereas vP658 did not produce any extracellular forms of **E**, but contained additional cDNA sequences encoding the NS2B protein. In the challenge experiments several dilutions of challenge virus were tested,. . . . dose of JEV. The analysis demonstrated that: (1) only those animals immunized with vP555 showed a strong immune response to **E**, and (2) a second inoculation resulted in a significant increase in reactivity to the **E** protein (FIG. 10).

DETD . . . induce neutralizing antibodies may be related to the fact that vP555 produces an extracellular particulate form of the structural proteins **E** and M. This SHA-like particle probably represents an empty JEV envelope that contains **E** and M folded and assembled into a configuration very similar to that found in the infectious JEV particle. Recombinants vP555. . . assembly of viral envelopes. Other investigators (see above) have not been able to detect the production of extracellular forms of **E** by cells expressing all three structural proteins (C, **prM**, and **E**) in the presence or absence of NS1 and NS2A. The inability of their recombinant viruses to produce particles similar to. . .

DETD . . . were obtained from GIBCO/BRL, Gaithersburg, MD, New England Biolabs, Beverly, MA; and Boehringer Mannheim Biochemicals, Indianapolis, IN. Klenow fragment of **E**. coli polymerase was obtained from Boehringer Mannheim Biochemicals. BAL-31 exonuclease and phage T4 DNA ligase were obtained from New England. . .

DETD . . . NcoI site (pos. 172,253) were removed by digestion of pSD419 with NcoI/SmaI followed by blunt ending with Klenow fragment of **E**. coli polymerase and ligation generating plasmid pSD476. A vaccinia right flanking arm was obtained by digestion of pSD422 with HpaI. . . ID NO:6/SEQ ID NO:7) ##STR3## generating pSD479. pSD479 contains an

initiation codon (underlined) followed by a BamHI site. To place **E.** coli Beta-galactosidase in the B13-B14 (u) deletion locus under the control of the u promoter, a 3.2 kb BamHI fragment. . .

DETD . . . at the pUC/vaccinia junction was destroyed by digestion of pSD478 with EcoRI followed by blunt ending with Klenow fragment of **E.** coli polymerase and ligation, generating plasmid pSD478E-. pSD478E- was digested with BamHI and HpaI and ligated with annealed synthetic oligonucleotides. . .

DETD . . . XbaI within vaccinia sequences (pos. 137,079) and with HindIII at the pUC/vaccinia junction, then blunt ended with Klenow fragment of **E.** coli polymerase and ligated, resulting in plasmid pSD483. To remove unwanted vaccinia DNA sequences to the right of the A26L. . . digestion with BglII (pos. 140,136) and with EcoRI at the pUC/vaccinia junction, followed by blunt ending with Klenow fragment of **E.** coli polymerase and ligation. The resulting plasmid was designated pSD489. The 1.8 kb ClaI (pos. 137,198)/EcoRV (pos. 139,048) fragment from. . .

DETD A 3.3 kb BglII cassette containing the **E.** coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD A 3.2 kb BglII/BamHI (partial) cassette containing the **E.** coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . were removed from the pUC/vaccinia junction by digestion of pSD466 with EcoRI/BamHI followed by blunt ending with Klenow fragment of **E.** coli polymerase and ligation. Recombination between vP708 and pSD467 resulted in recombinant: vaccinia deletion mutant, vP723, which was isolated as. . .

DETD To provide a substrate for the deletion of the [C7L-K1L] gene cluster from vaccinia, **E.** coli Beta-galactosidase was first inserted into the vaccinia M2L deletion locus (Guo et al., 1990) as follows. To eliminate the. . . unique BglII site inserted into the M2L deletion locus as indicated above. A 3.2 kb BamHI (partial)/BglII cassette containing the **E.** coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the 11 kDa promoter (Bertholet et al., 1985) was. . .

DETD . . . deleted for vaccinia genes [C7L-K1L] was assembled in PUCS cut with SmaI, HindIII and blunt ended with Klenow fragment of **E.** coli polymerase. The left flanking arm consisting of vaccinia HindIII C sequences was obtained by digestion of pSD420 with XbaI (pos. 18,628) followed by blunt ending with Klenow fragment of **E.** coli polymerase and digestion with BglII (pos. 19,706). The right flanking arm consisting of vaccinia HindIII K sequences was obtained. . .

DETD . . . coding sequences, pSD518 was digested with BamHI (pos. 65,381) and HpaI (pos. 67,001) and blunt ended using Klenow fragment of **E.** coli polymerase. This 4.8 kb vector fragment was ligated with a 3.2 kb SmaI cassette containing the **E.** coli Beta-galactosidase gene (Shapira et al., 1983) under the control of the vaccinia 11 kDa promoter (Bertholet et al., 1985;. . .

DETD . . . mutagenized expression cassette contained within pRW837 was derived by digestion with HindIII and EcoRI, blunt-ended using the Klenow fragment of **E.** coli DNA polymerase in the presence of 2mM dNTPs, and inserted into the SmaI site of pSD513 to yield pRW843.. . .

DETD . . . into pRW843 (containing the measles HA gene). Plasmid pRW843 was first digested with NotI and blunt-ended with Klenow fragment of **E.** coli DNA polymerase in the presence of 2mM dNTPs. The resulting plasmid, pRW857, therefore contains the measles virus F and. . .

DETD . . . of JEV. First strand cDNA synthesis was primed from a synthetic oligonucleotide complementary to bases 986 to 1005 of the **E** coding region of JEV (FIG. 17A and B) (SEQ ID NO:52). The double-stranded cDNA was ligated to synthetic oligonucleotides containing. . . Biolabs, Beverly, MA), inserted into phosphatase treated EcoRI-cleaved pBR322 (New England Biolabs), and the resulting DNA was used to transform **E.** coli strain DH5 cells (GIBCO/BRL). Plasmids were analyzed by restriction enzyme digestion and a plasmid (pC20) containing cDNA corresponding to 81 nucleotides of non-coding RNA and the C and **prM** coding regions was identified. pC20 was digested at the linker sites with EcoRI and at an internal DraI site situated 28 bp 5' of the ATG initiation codon and the resulting fragment containing the C and **prM** coding regions was inserted into SmaI-EcoRI digested pUC18, creating plasmid, pDr20. The sequence of the C coding region of pC20, combined with an updated sequence of the **prM**, **E**, NS1, NS2A, and NS2B coding regions of the Nakayama strain of JEV is presented in FIG. 17A and B (SEQ. . .

DETD . . . the XhoI and AccI fragment of JEV2 (FIG. 1) containing the plasmid origin and JEV cDNA encoding the carboxy-terminal 40% **prM** and amino-terminal two thirds of **E** (nucleotides 603 to 2124), generating plasmid JEV20 containing JE sequences from the ATG of C through the SacI site (nucleotide 2124) found in the last third of **E.**

DETD . . . 1) in which TTTTTGT nucleotides 1304 to 1310 were changed to TCTTTGT), containing JE sequences from the last third of **E** through the first two amino acids of NS2B (nucleotides 2124 to 4126), the plasmid

origin and vaccinia sequences, was ligated. . .

DETD . . . end] generated plasmid JEV25 which contains JE cDNA extending from the SacI site (nucleotide 2124) in the last third of **E** through the carboxy-terminus of **E**. The SacI-EagI fragment from JEV25 was ligated to the SacI-EagI fragment of JEV8 (containing JE cDNA encoding 15 aa C, **prM** and amino-terminal two thirds of **E** nucleotides 337 to 2124, the plasmid origin and vaccinia sequences) yielding plasmid JEV26. A unique SmaI site preceding the ATG. . .

DETD . . . or HpaI-HindIII fragment from JEV7 (FIG. 2) yielded JEV29 [containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A (nucleotides 2293 to 4125)] and JEV30 [containing a SmaI site followed by JE cDNA encoding 30 aa **E**, NS1, NS2A, NS2B (nucleotides 2293 to 4512)].

DETD Recombinant vP825 encoded the capsid protein C, structural protein precursor **prM**, the structural glycoprotein **E**, the nonstructural glycoprotein NS1, and the nonstructural protein NS2A (McAda et al., 1987). Recombinant vP829 encoded the putative 15 aa signal sequence preceding the amino-terminus of **prM**, as well as **prM**, and **E** (McAda et al., 1987). Recombinant vP857 contained a cDNA encoding the 30 aa hydrophobic carboxy-terminus of **E**, followed by NS1 and NS2A. Recombinant vP864 contained a cDNA encoding the same proteins as vP857 with the addition of NS2B. In recombinants vP825 and vP829 a potential vaccinia virus early transcription termination signal in **E** (TTTTTGT; nucleotides 1399-1405) was modified to TCTTTGT without altering the aa sequence. This change was made in an attempt to increase the level of expression of **E** since this sequence has been shown to increase transcription termination in in vitro transcription assays (Yuen et al., 1987).

DETD **E** and **prM** Were Properly Processed When Expressed by Recombinant Vaccinia Viruses

DETD Pulse-chase experiments demonstrate that proteins identical in size to **E** were synthesized in cells infected with all recombinant vaccinia viruses containing the **E** gene (Table 3). In the case of cells infected with JEV, vP555 and vP829, an **E** protein that migrated slower in SDS-PAGE was also detected in the culture fluid harvested from the infected cells (Table 3). This extracellular form of **E** produced by JEV- and vP555-infected cells contained mature N-linked glycans (Mason, 1989; Mason et al., 1991), as confirmed for the extracellular forms of **E** produced by vP829-infected cells. Interestingly, vP825, which contained the C coding region in addition to **prM** and **E** specified the synthesis of **E** in a form that is not released into the extracellular fluid (Table 3). Immunoprecipitations prepared from radiolabeled vaccinia-infected cells using a MAb specific for M (and **prM**) revealed that **prM** was synthesized in cells infected with vP555, vP825, and vP829, and M was detected in the culture fluid of cells. . .

DETD . . . . fluids (Table 3). This result indicated that vP829 infected cells produced extracellular particles similar to the empty viral envelopes containing **E** and M which are observed in the culture fluids harvested from vP555 infected cells (FIG. 9).

DETD Pre-challenge sera pooled from selected animals in each group were tested for their ability to immunoprecipitate radiolabeled **E** and NS1. The results of these studies (Table 3) demonstrated that: (1) the following order of immune response to **E** vP829>vP555>vP825, (2) all viruses encoding NS1 and NS2A induced antibodies to NS1, and (3) all immune responses were increased by. . . sera collected from these animals (Table 4) confirmed the results of the immunoprecipitation analyses, showing that the immune response to **E** as demonstrated by RIP correlated well with these other serological tests (Table 4).

DETD TABLE 3

Characterization of proteins expressed by vaccinia recombinants and their immune responses

	vP555	vP829	vP825	vP857	vP864
Proteins expressed					
Intracellular	**prM,E** NS1	**prM,E**	**prM,E** NS1	NS1	NS1
secreted	M,**E**,NS1	M,**E**	NS1	NS1	NS1
Particle formation	+	+	-	-	-
Immune response					
single	**E**	**E**	NS1	NS1	NS1
double	**E**,NS1	**E**	**E**,NS1	NS1	NS1

single = single inoculation with 10^7 pfu vaccinia recombinants (ip)
double = two inoculations with 10^7 pfu vaccinia. . .

DETD . . . isolated and ligated to a SacI (JEV nucleotide 2125) to EagI fragment of JEV25 (containing the remaining two thirds of **E**,

translation stop and T5NT) generating JEV36. JEV36 was transfected into vP866 (NYVAC) infected cells to generate the vaccinia recombinant vP923. . .

DETD Plasmid YFO containing YF cDNA encoding the carboxy-terminal 80% **prM, E** and amino-terminal 80% NS1 (nucleotides 537-3266) was derived by cloning an AvaI to NsiI fragment of YF cDNA (nucleotides 537-1658). . . and KpnI digested IBI25 (International Biotechnologies, Inc., New Haven, CT). Plasmid YF1 containing YF cDNA encoding C and amino-terminal 20% **prM** (nucleotides 119-536) was derived by cloning a RsaI to AvaI fragment of YF cDNA (nucleotides 166-536) and annealed oligos SP46. . . and YF nucleotides 119-165) into AvaI and HindIII digested IBI25. Plasmid YF3 containing YF cDNA encoding the carboxy-terminal 60% of **E** and amino-terminal 25% of NS1 was generated by cloning an ApaI to BamHI fragment of YF CDNA (nucleotides 1604-2725) into. . . cDNA (nucleotides 4339-4940) into SacI and XbaI digested IBI25. Plasmid YF13 containing YF cDNA encoding the carboxy-terminal 25% of C, **prM** and amino-terminal 40% of **E** was derived by cloning a BalI to AnaI fragment of YF cDNA (nucleotides 384-1603) into ApaI and SmaI digested IBI25.

DETD . . . gene in YF1 (TTTTTCT nucleotides 263-269 and TTTTTGT nucleotides 269-275) to (SEQ ID NO:35) TTCTTCTTCTTGT creating plasmid YF1B, in the **E** gene in YF3 (nucleotides 1886-1893 TTTTTTGT to TTCTTTGT 189 aa from the carboxy-terminus and nucleotides 2429-2435 TTTTTGT to TTCTTGT 8. . . YF3C (nucleotides 1965-2725) was exchanged for the corresponding fragment of YF3B generating YF4 containing YF CDNA encoding the carboxy-terminal 60% **E** and amino-terminal 25% NS1 (nucleotides 1604-2725) with both mutagenized transcription termination signals. An ApaI to BamHI fragment from YF4 (nucleotides 1604-2725) was substituted for the equivalent region in YFO creating plasmid YF6 containing YF cDNA encoding the carboxy-terminal 80% **prM, E** and amino-terminal 80% NS1 (nucleotides 537-3266) with both mutagenized transcription termination signals. Plasmid YF6 was digested with EcoRV within the. . .

DETD . . . mutagenesis described above was used to insert XhoI and ClaI sites preceding the ATG 17 aa from the carboxy-terminus of **E** (nucleotides 2402-2404) in plasmid YF3C creating YF5, to insert XhoI and ClaI sites preceding the ATG 19 aa from the carboxy-terminus of **prM** (nucleotides 917-919) in plasmid YF13 creating YF14, to insert an XhoI site preceding the ATG 23 aa from the carboxy-terminus of **E** (nucleotides 2384-2386) in plasmid YF3C creating plasmid YF25, and to insert an XhoI site and ATG (nucleotide 419) in plasmid. . .

DETD . . . YF5 (nucleotides 1604-2725) was exchanged for the corresponding region of YFO creating YF7 containing YF cDNA encoding the carboxy-terminal 80% **prM, E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at 2402 (17 aa from the carboxy-terminus of **E**) and a mutagenized transcription termination signal at 2429-2435 (8 aa from the carboxy-terminus of **E**). The ApaI to BamHI fragment from YF25 (nucleotides 1604-2725) was exchanged for the corresponding region of YFO generating YF26 containing YF cDNA encoding the carboxy-terminal 80% **prM, E** and amino-terminal 80% NS1 (nucleotides 537-3266) with an XhoI site at nucleotide 2384 (23 aa from the carboxy-terminus of **E**) and mutagenized transcription termination signal at 2428-2435 (8 aa from the carboxy-terminus of **E**).

DETD . . . YF14 (nucleotides 537-1603) was substituted for the corresponding region in YF6 generating YF15 containing YF cDNA encoding the carboxy-terminal 80% **prM, E** and amino-terminal 80% NS1 (nucleotides 537-3266) with XhoI and ClaI sites at nucleotide 917 (19 aa from the carboxy-terminus of **prM**) and two mutagenized transcription termination signals. YF6 was digested within IBI25 with EcoRV and within YF at nucleotide 537 with. . .

DETD . . . from YF12 encoding carboxy-terminal 20% NS1, NS2A and NS2B (nucleotides 3267-4569), XhoI to KpnI fragment from YF1S encoding 19 aa **prM, E** and amino-terminal 80% NS1 (nucleotides 917-3266) and XhoI-SmaI digested pHES4 were ligated generating YF23. An XhoI to BamHI fragment from YF26 encoding 23 aa **E**, amino-terminal 25% NS1 (nucleotides 2384-2725) was ligated to an XhoI to BamHI fragment from YF23 (containing the carboxy-terminal 75% NS1,. . .

DETD XhoI-SmaI digested pHES4 was ligated to a purified XhoI to XpnI fragment from YF7 encoding 17 aa **E** and amino-terminal 80% NS1 (nucleotides 2402-3266) plus a KpnI to SmaI fragment from YF10 encoding the carboxy-terminal 20% NS1 and NS2A (nucleotides 3267-4180) creating YF18. An XhoI to BamHI fragment from YF2 encoding C, **prM, E** and amino-terminal 25% NS1 (nucleotides 119-2725) was ligated to a XhoI to BamHI fragment of YF18 (containing the carboxy-terminal 75%. . . the origin of replication and vaccinia sequences) generating YF20. A XhoI to BamHI fragment from YF46 encoding 21 aa C, **prM, E** and amino-terminal 25% NS1 (nucleotides 419-2725) was ligated to the XhoI to BamHI fragment from YF18 generating YF47. Oligonucleotide SP46. . .

DETD Radiolabeled cell lysates and culture fluids were harvested and the viral proteins were immunoprecipitated with monoclonal antibodies to YF

E and NS1 and separated in SDS-containing polyacrylamide gels exactly as described by Mason (1989).

DETD . . . NS1 and NS2A (Rice et al., 1985). Recombinant vP729 encoded the putative 19-aa signal sequence preceding the N terminus of **E**, **E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP764 encoded C, **prM**, **E**, NS1, NS2A and NS2B (Rice et al., 1985). Recombinant vP766 encoded C, **prM**, **E**, NS1 and NS2A (Rice et al., 1985). Recombinant vP869 encoded the putative 21-aa signal sequence preceding the N terminus of the structural protein precursor **prM**, **prM E**, NS1 and NS2A (Rice et al., 1985).

DETD **E** Protein Expression By Recombinant Vaccinia Virus

DETD Pulse-chase experiments in HeLa cells demonstrated that a protein identical in size to YF17D **E** was synthesized in cells infected with vP869 and secreted into the culture fluid (Table 7). Under the same conditions of labeling, no intracellular or extracellular **E** was detected in cultures infected with vP766, vP729 or the control vaccinia virus vP457 (Table 7).

DETD Continuous label experiments in Vero cells demonstrated that a protein identical in size to the **E** protein expressed by vP869 was expressed in cultures infected with vP766 and vP729 (Table 7). These results suggest that the **E** protein produced by vP869 infected cells is present in a form in which it is more stable than the **E** protein expressed by vP766 or vP729. YF17D has previously been shown to produce a more labile **E** protein than other YF isolates (Cane et al. 1989).

DETD . . . immunization with YF17D. Pre-challenge sera pooled from selected animals in each group were tested for their ability to immunoprecipitate radiolabeled **E** and NS1 proteins and for the presence of Neut and HAI antibodies. As shown in Table 9 only vP869 and YF17D immunized mice responded to **E** protein, the response was increased by a second inoculation. Mice immunized twice with vP729, vP725 or vP766 produced antibody to. . .

DETD TABLE 7

Characterization of proteins expressed by vaccinia recombinants and YF17D

	17D	vP869	vP729	vP725	vP766	vP457
YF Proteins Expressed Intracellular	**E**,NS1	**E**	**E**,NS1	NS1	**E**,NS1	NONE
Secreted	**E**,NS1	**E**	NS1	NS1	NONE	NONE
Extracellular HA Activity	YES	YES	NO	NO	NO	NO

DETD TABLE 9

Pre-challenge Radioimmunoprecipitation

Immunizing Virus	One Inoculation		Two Inoculations	
	Anti-**E**	Anti-NS1	Anti-**E**	Anti-NS1
vP457	-	-	-	-
vP725				+
vP729				+
vP766				+
vP869	+	-	++	-
17D	+	-	++	-

DETD A XhoI to SmaI fragment from YF47 (nucleotides 419-4180) containing YF CDNA encoding 21 amino acids C, **prM**, **E**, NS1, NS2A (with a base missing in NS1 nucleotide 2962) was ligated to XhoI-SmaI digested SPHA-H6 (HA region donor plasmid). . . (nucleotide 3262) and a 6700 bp fragment isolated (containing the plasmid origin of replication, vaccinia sequences, 21 amino acids C, **prM**, **E**, amino-terminal 3.5% NS1, carboxy-terminal 23% NS1, NS2A) and ligated to a SacI-Asp718 fragment from YF18 (containing the remainder of NS1. . . site in YF51 were removed using oligonucleotide-directed double-strand break mutagenesis (Mandecki, 1986) creating YF50 encoding YF 21 amino acids C, **prM**, **E**, NS1, NS2A in the HA locus donor plasmid. YF50 was transfected into vP866 (NYVAC) infected cells generating the recombinant vP984. . .

DETD . . . double-strand break mutagenesis creating YF49. Oligonucleotide-directed mutagenesis (Kunkel, 1985) was used to insert a SmaI site at the carboxy-terminus of **E** (nucleotide 2452) in YF4 creating YF16. ApaI-SmaI fragment of YF49 (containing the plasmid origin of replication, vaccinia sequences and YF CDNA encoding 21 amino acids

C, **prM**, and amino-terminal 43% **E**) was ligated to an ApaI-SmaI fragment from YF16 (nucleotides 1604-2452 containing the carboxy-terminal 57% **E**) generating YF53 containing 21 amino acids C, **prM**, **E** in the HA locus donor plasmid. YF53 was transfected into vP866 (NYVAC) infected cells generating the recombinant vP1003 (FIG. 19).. . .

DETD . . . digested pUC8. An EcoRI-HindIII fragment from DEN1 (nucleotides 2559-3745) and SacI-EcoRI fragment of DEN CDNA encoding the carboxy-terminal 36% of **E** and amino-terminal 16% NS1 (nucleotides 1447-2559, Mason et al., 1987B) were ligated to HindIII-SacI digested IBI24 (International Biotechnologies, Inc., New Haven, Conn.) generating DEN3 encoding the carboxy-terminal 64% **E** through amino-terminal 45% NS2A with a base missing in NS1 (nucleotide 2467).

DETD . . . an AvaI-SacI fragment of DEN cDNA (nucleotides 424-1447 Mason et al., 1987B) generating DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E**.

DETD Plasmid DEN6 containing DEN cDNA encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579 with nucleotide 2467 present Mason et al., 1987B) was derived by cloning a SacI-XhoI. . . Biotechnologies, Inc., New Haven, Conn.). Plasmid DEN15 containing DEN cDNA encoding 51 bases of the DEN 5' untranslated region, C, **prM** and amino-terminal 36% **E** was derived by cloning a HindIII-SacI fragment of DEN cDNA (nucleotides 20-1447, Mason et al., 1987B) into HindIII-SacI digested IBI25.. . .

DETD . . . mutagenesis (Kunkel, 1985) was used to change potential vaccinia virus early transcription termination signals (Yuen et al., 1987) in the **prM** gene in DEN4 29 aa from the carboxy--terminus (nucleotides 822-828 TTTTTCT to TATTTCT) and 13 aa from the carboxy-terminus (nucleotides. . .

DETD . . . (nucleotide 4102) in plasmid DEN23 creating DEN24, to insert a SmaI site and ATG 15 aa from the carboxy-terminus of **E** in DEN7 (nucleotide 2348) creating DEN10, to insert an EagI and HindIII site at the carboxy-terminus of NS2B (nucleotide 4492). . .

DETD . . . DEN7 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN19 containing DEN CDNA encoding the carboxy-terminal 64% **E** and amino-terminal 45% NS2A (nucleotides 1447-3745) with nucleotide 2467 present and the modified transcription termination signal (nucleotides 2448-2454). A XhoI-XbaI. . .

DETD . . . of the H6 promoter and DEN nucleotides 68-494) was ligated to a HindIII-PstI fragment from DEN47 (encoding the carboxy-terminal 83% **prM** and amino-terminal 36% of **E** nucleotides 494-1447 and plasmid origin of replication) generating DEN17 encoding C, **prM** and amino-terminal 36% **E** with part of the H6 promoter and EcoRV site preceding the amino-terminus of C. A HindIII-BlII fragment from DEN17 encoding the carboxy-terminal 13 aa C, **prM** and amino--terminal 36% **E** (nucleotides 370-1447) was ligated to annealed oligonucleotides SP111 and SP112 (containing a disabled HindIII sticky end, EcoRV site to -1. . . a BglII sticky end) creating DEN33 encoding the EcoRV site to -1 of the H6 promoter, carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**.

DETD . . . digested pTP15 (Mason et al., 1991) was ligated to a SmaI-SacI fragment from DEN4 encoding the carboxy-terminal 11 aa C, **prM** and amino-terminal 36% **E** (nucleotides 377-1447) and SacI-EagI fragment from DEN3 encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A generating DENL. The SacI-XhoI fragment from DEN7 encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 (nucleotides 1447-2579) was ligated to a BstEII-SacI fragment from DEN47 (encoding the carboxy-terminal 55% **prM** and amino-terminal 36% **E** (nucleotides 631-1447) and a BstEII-XhoI fragment from DENL (containing the carboxy-terminal 11 aa C, amino-terminal 45% **prM**, carboxy-terminal 82% NS1, carboxy-terminal 45% NS2A, origin of replication and vaccinia sequences) generating DEN8. A unique SmaI site (located between. . .

DETD An EcoRV-SacI fragment from DEN17 (positions -21 to -1 H6 promoter DEN nucleotides 68-1447) encoding C, **prM** and amino-terminal terminal 36% **E**) was ligated to an EcoRV-SacI fragment of DEN8VC (containing vaccinia sequences, H6 promoter from -21 to -124, origin of replication and amino-terminal 64% **E**, NS1, amino-terminal terminal 45% NS2A nucleotides 1447-3745) generating DEN18. A XhoI-EagI fragment from DEN25 encoding the carboxy-terminal 82% NS1 and. . . (nucleotides 2579-4102) was ligated to an XhoI-EagI fragment of DEN18 (containing the origin of replication, vaccinia sequences and DEN C **prM**, **E** and amino-terminal 18% NS1 nucleotides 68-2579) generating DEN26. An EcoRV-SacI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN nucleotides 377-1447 encoding 11aaC, **prM** and amino-terminal 36% **E**) was ligated to an EcoRV-SacI fragment of DEN26 (containing the origin of replication, vaccinia sequences and DEN region encoding the carboxy-terminal 64% **E**, NS1 and NS2A with a base missing in NS1 at nucleotide 2894) generating DEN32. DEN32 was transfected into vP410

infected. . .

DETD . . . DEN10 (nucleotides 1447-2579) was substituted for the corresponding region in DEN3 generating DEN11 containing DEN CDNA encoding the carboxy-terminal 64% **E**, NS1 and amino-terminal 45% NS2A with a SmaI site and ATG 15 aa from the carboxy-terminus of **E**. A SmaI-EagI fragment from DEN11 (encoding the carboxy-terminal 15 aa **E**, NS1 and amino-terminal 45% NS2A nucleotides 2348-3745) was ligated to SmaI-EagI digested pTP15 generating DEN12.

DETD An EcoRV-XhoI fragment from DEN8VC (positions -21 to -1 H6 promoter DEN nucleotides 377-2579 encoding the carboxy-terminal 11 aa C, **prM E**, amino-terminal 18% NS1) was ligated to an EcoRV-XhoI fragment from DEN31 (containing the origin of replication, vaccinia sequences and DEN. . . An EcoRV-SacI fragment from DEN33 (positions -21 to -1 H6 promoter DEN nucleotides 350-1447 encoding the carboxy-terminal 20 aa C, **prM** and amino-terminal 36% **E**) and a SacI-XhoI fragment from DEN32 (encoding the carboxy-terminal 64% **E** and amino-terminal 18% NS1 nucleotides 1447-2579) were ligated to the EcoRV-SacI fragment from DEN31 described above generating DEN34. DEN34 was. . .

DETD Construction of Vaccinia Insertion Vector Containing DEN Type 1 20 aaC, **prM, E**

DETD A 338bp fragment encoding the carboxy-terminal 23% **E** (nucleotides 2055-2392, Mason et al., 1987b) TGA stop codon T5NT vaccinia early transcription termination signal (Yuen et al., 1987) and. . . and cloned into HindIII/BamHI digested IBI25 generating DEN36. DEN34 was digested with EcoRV (within the H6 promoter) and HindIII within **E** (DEN nucleotide 2061; Mason et al., 1987b) and a 1733 bp fragment (containing EcoRV to -1 H6 promoter, 20 aaC, **prM** and amino-terminal 77% **E**) was isolated. DEN36 was digested with HindIII and EclXI and a 331 bp fragment isolated (containing DEN nucleotides 2062-2392 TGA. . . generating plasmid DEN38. Plasmid DEN38 can be transfected into vaccinia infected cells to generate a recombinant encoding DEN 20 aaC, **prM** and **E**.

DETD This example describes the development of canarypox recombinant vCP107 encoding JEV 15aaC, **prM**, **E**, NS1, NS2A and a canarypox donor plasmid (JEVCPC5) encoding 15aaC, **prM**, **E**.

DETD Construction of Insertion Vector Containing JEV 15aaC, **prM**, **E**, NS1, NS2A

DETD Construction of pRW838 is illustrated below (FIG. 23). Oligonucleotides A through **E**, which overlap the translation initiation codon of the H6 promoter with the ATG of rabies G, were cloned into pUC9 as pRW737. Oligonucleotides A through **E** contain the H6 promoter, starting at NruI, through the HindIII site of rabies G followed by BglII. Sequences of oligonucleoties A through **E** are: ##STR16## The diagram of annealed oligonucleotides A through **E** is as follows: ##STR17##

DETD Oligonucleotides A through **E** were kinased, annealed (95° C. for 5 minutes, then cooled to room temperature), and inserted between the PvuII sites of. . .

DETD . . . promoter, plasmid origin of replication and C5 flanking arms isolated. Plasmid JEVL14VC containing JEV cDNA encoding 15 amino acids C, **prM**, **E**, NS1, NS2A in a vaccinia virus donor plasmid (FIG. 1) (nucleotides 337-4125, FIG. 17A and B) (SEQ ID NO:52) was. . . JEVCP1 was transfected into ALVAC infected primary CEF cells to generate the canarypox recombinant vCP107 encoding 15 amino acids C, **prM**, **E**, NS1, NS2A (FIG. 18).

DETD Construction of C5 Insertion Vector Containing JEV 15aac, **prM**, **E**

DETD . . . annealed oligonucleotides SP131 (SEQ ID NO:75) and SP132 (SEQ ID NO:76) (containing a SphI sticky end, T nucleotide completing the **E** coding region, translation stop, a vaccinia early transcription termination signal (AT5AT; Yuen and Moss, 1987), a second translation stop, and XbaI sticky end) generating plasmid JEVCP5 which encodes 15 amino acids C, **prM** and **E** under the control of the H6 promoter between C5 flanking arms. JEVCP5 can be transfected in ALVAC or ALVAC recombinant infected cells to generate a recombinant encoding JEV 15 aa C, **prM** and **E**. ##STR19## Example 15--CONSTRUCTION OF ALVAC RECOMBINANT EXPRESSING YFV PROTEINS Construction of Canarypox Insertion Vector

DETD . . . and SmaI and ligated to a 3772 bp XhoI-SmaI fragment from YF51 (nucleotides 419-4180 encoding YF 21 amino acids C, **prM**, **E**, NS1, NS2A) generating YF52. The 6 bp corresponding to the unique XhoI site in UP52 were removed using oligonucleotide-directed double-strand. . . YFCP3. YFCP3 was transfected into ALVAC infected primary CEF cells to generate the canarypox recombinant vCPI27 encoding 21 aa C, **prM**, **E**, NS1, NS2A (FIG. 19).

DETD Construction of C3 Insertion Vector Containing YFV 21 aa C, **prM**, **E**

DETD . . . 8344 bp fragment isolated (containing the plasmid origin of replication, canarypox DNA and YF cDNA encoding 21 amino acids C, **prM**, and amino-terminal 57% **E**) and ligated to a ApaI to SmaI fragment from YF16 (nucleotides 1604-2452 containing the carboxy-terminal 43% **E**) generating YF54. The 6 bp corresponding to the unique XhoI site in YF54 were removed as described above creating YFCP4 containing YF cDNA encoding 21 amino acids C, **prM**, and **E**. YFCP4 can be transfected into

ALVAC or ALVAC recombinant infected cells to generate a recombinant encoding YFV 21 aa C, **prM**, **E**.

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CLM What is claimed is:

1. A recombinant poxvirus comprising DNA coding for at least one flavivirus structural protein, wherein the flavivirus is **Yellow Fever virus** or Dengue virus and the poxvirus is selected from the group consisting of: an avipox virus, a vaccinia virus wherein. . . . . recombinant poxvirus of claim 1 wherein the DNA encodes protein M or a precursor to protein M, and flavivirus proteins **E**, NS1 and NS2A.

7. The recombinant poxvirus of claim 1 wherein the flavivirus is **Yellow Fever virus**.

=> d his

(FILE 'HOME' ENTERED AT 15:35:18 ON 23 FEB 2004)

FILE 'USPATFULL' ENTERED AT 15:35:57 ON 23 FEB 2004
L1 661 S YELLOW FEVER VIRUS
L2 59 S L1 AND (YELLOW FEVER VIRUS/CLM)
L3 17 S L2 AND (PRM AND E)
L4 4 S L3 AND (PRM/CLM)
L5 13 S L3 NOT L4
L6 7 S L3 AND E/CLM

=> d l6,cbib,kwic,1-6

L6 ANSWER 1 OF 7 USPATFULL on STN
2003:276776 Use of flavivirus for the expression of protein epitopes and development of new live attenuated vaccine virus to immune against flavivirus and other infectious agents.
Bonaldo, Mirna C., Rio de Janeiro, BRAZIL
Galler, Ricardo, Rio de Janeiro, BRAZIL
Freire, Marcos da Silva, Rio de Janeiro, BRAZIL
Garrat, Richard C., Sao Paulo, BRAZIL
US 2003194801 A1 20031016
APPLICATION: US 2003-275707 A1 20030410 (10)
WO 2002-BR36 20020308
PRIORITY: GB 2001-5877 20010309
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
SUMM . . . was determined on the genome of the YF 17D-204 vaccine strain virus by Rice et al (Rice C. M.; Lenches, **E.**; Eddy, S. R.; Shin, S. J.; Sheets, R. L. and Strauss, J. H. 1985. "Nucleotide sequence of **yellow fever virus**: implications for flavivirus gene expression and evolution". Science. 229: 726-733).
SUMM . . . by proteolytic processing to generate 10 virus-specific polypeptides. From the 5' terminus, the order of the encoded proteins is: C; **prM**/M; **E**; NS1; NS2A; NS2B; NS3; NS4A; NS4B and NS5. The first 3 proteins constitute the structural proteins, that is, form the virus together with the packaged RNA molecule and were named capsid (C, 12-14 kDa), membrane (M, and its precursor **prM**, 18-22 kDa) and envelope (**E**,52-54 kDa) all being encoded in the first quarter of the genome. The remainder of the genome codes for the nostructural. . .
SUMM . . . RNA accumulation and mouse neurovirulence. Virology 222, 159-168; Muylaert I R; Galler R, Rice C M 1997. Genetic analysis of **Yellow Fever virus** NS1 protein: identification of a temperature-sensitive mutation which blocks RNA accumulation. J. Virol 71, 291-298; Lindenbach B D, Rice C. . . NS4A as a determinant of replicase function J. Virol. 73, 4611-4621; Lindenbach B D, Rice C M 1997. trans-complementation of **yellow fever virus** NS1 reveals a role in early RNA replication J. Virol. 71, 9608-9617).
SUMM . . . Bazan J F, Fletterick R J, Rice C M 1990b. Evidence that the N-terminal domain of nonstructural protein NS3 from **yellow fever virus** is a serine protease responsible for site-specific cleavages in the viral polyprotein. Proc.Natl.Acad.Sci. USA 87, 8898-8902; Falgout B, Pethel M,. . . Virol. 69, 1995-2003; Yamshichikov V F; Trent, D W, Compans R W 1997. Upregulation of signalase processing and induction of **prM-E** secretion by the flavivirus NS2B-NS3 protease: roles of protease components. J. Virol. 71, 4364-4371; Stocks C **E**, Lobigs M 1998. Signal peptidase cleavage at the flavivirus C-**prM** junction: dependence on the viral NS2B-3 protease for efficient processing requires determinants in C, the signal peptide and **prM**. J. Virol. 72, 2141-2149). It also contains nucleotide triphosphatase/helicase activities (Gorbalenya A **E**, Koonin **E** V, Donchenko A P, Blinov V M 1989. Two related superfamilies of putative helicases involved in replication, recombination repair and. . . J, Hahn C S, Galler R, C M Rice 1990a. Flavivirus genome organization, expression and evolution. Ann.Rev.Microbiol. 44, 649-688; O'Reilly **E** K, Kao C C 1998. Analysis of RNA-dependent RNA polymerase structure and function as guided by known polymerase structures and. . . predictions of secondary structure. Virology 252, 287-303) and exhibits RNA-dependent RNA polymerase activity (Steffens S, Thiel H J, Behrens S **E** 1999. The RNA-dependent RNA polymerases of different members of the family Flaviviridae exhibit similar properties in vitro. J. Gen. Virol.. . . of cis and trans acting elements in flavivirus RNA replication have been identified (Kromykh A A, Sedlak P L, Westaway **E** G 2000. cis- and trans-acting elements in flavivirus RNA replication. J. Virol. 74, 3253-3263).
SUMM . . . the active viral protease complex (Chambers T J, Nestorowicz A, Amberg S M, Rice C M 1993. Mutagenesis of the **yellow fever virus** nonstructural polyportein: a catalitically active NS3 proteinase domain and NS2B are required for cleavages at dibasic sites. J. Virol. 65,. . .
SUMM [0008] Two strains of **yellow fever virus** (YF), isolated in 1927,

gave rise to the vaccines to be used for human immunization. One, the Asibi strain, was. . .

SUMM . . . and Smith (Theiler M and Smith H H. 1937. The effect of prolonged cultivation in vitro upon the pathogenicity of **yellow fever virus**. J Exp Med. 65, 767-786) showed that, at this stage, there was a marked reduction in viral viscero and neurotropism. . . absence of adverse reactions and seroconversion to YF in 2 weeks (Theiler M, Smith H H 1937. The use of **yellow fever virus** modified by in vitro cultivation for human immunization J. Exp. Med 65:787-800).

SUMM . . . various countries in Africa. Fox, J. P. et al (Fox, F. P. and Penna, H. A. (1943). Behavior of 17D **yellow fever virus** in Rhesus monkeys. Relation to substrain, dose and neural or extraneural inoculation. Am. J. Hyg. 38: 52-172) described the preparation. . .

SUMM . . . in SW13 cells and used for cDNA cloning and sequence analyses. These 17D-204 are named C-204 (Rice, C. M.; Lenches, **E**.; Eddy, S. R.; Shin, S. J.; Sheets, R. L. and Strauss, J. H. (1985). "Nucleotide sequence of **yellow fever virus**: implications for flavivirus gene expression and evolution". Science. 229: 726-733) and F-204 (Despres, P.; Cahour, A.; Dupuy, A.; Deubel, V.;. . .

SUMM . . . N, Post, P R, Carvalho, R, Ferreira I I, Rice C M and Galler, R. 1995. Complete nucleotide sequence of **yellow fever virus** vaccine strains 17DD and 17D-213. Virus Res. 35 :35-41) and was the subject of sequence analysis together with 17DD (at. . . C. S.; Dalrymple, J. M.; Strauss, J. H. and Rice, C. M. (1987). "Comparison of the virulent Asibi strain of **yellow fever virus** with the 17D vaccine strain derived from it". Proc. Natl. Acad. Sci. USA. 84: 2029-2033; 17D-204 strain, C-204: Rice. C. M.; Lenches, **E**. M.; Eddy, S. R.; Shin, S. J.; Sheets, R. L. and Strauss, J. H. (1985). "Nucleotide sequence of **yellow fever virus**: implications for flavivirus gene expression and evolution". Science. 229: 726-733; F-204: Despres, P.; Cahour, R.; Dupuy, A.; Deubel, V.; Bouloy,. . . M.; Digoutte, J. P. and Girard, M. (1987). "High genetic stability of the coding region for the structural proteins of **yellow fever virus** strain 17D". J. Gen. Virol. 68: 2245-2247). FIG. 1 depicts the passage history of the original YF Asibi strain and. . .

SUMM . . . scattered along the genome, 26 are silent mutations and 22 led to amino acid substitutions. The alterations noted in the **E** protein are important because it is the main target for humoral neutralizing response, i.**e**., it is the protein where hemagglutination and neutralization epitopes are located, and it mediates cell receptor recognition and cell penetration, therefore targeting the virus to specific cells. Importantly, **E** protein accumulate the highest ratio of nonconservative to conservative amino acid changes. Altogether, eleven nucleotide substitutions were observed in the **E** protein gene leading to 8 amino acid changes at positions 52, 170, 173, 200, 299, 305, 380 and 407 (respectively. . .

SUMM . . . 52 (G→R) and 200 (K→T) are located at the base of domain II in the 3-D structure proposed for the **E** protein of Flaviviruses (Rey, F. A.; Heinz F. X.; Mandl, C.; Kunz, C and Harrison, S. C. (1995). "The envelope. . . (Mandl, M. W.; Guirakhoo, F.; Holzmann, H.; Heinz, F. X. and Kunz, C. (1989). "Antigenic structure of the flavivirus envelope **E** protein at the molecular level using tick-borne encephalitis virus as a model". J. Virol. 63: 564-571). This domain II is. . .

SUMM [0015] Alterations at amino acids 170 and 173 in domain I of the **E** protein in the 3-D structure map very close to the position that a neutralization epitope was identified for tick-borne encephalitis (TBE) virus (see Mandl, C. W. et al, 1989). A mutation at position 171 of TBE virus **E** protein was shown to affect the threshold of fusion-activating conformational change of this protein and the 2 changes observed for. . . et al (Ryman K D, Xie H, Ledger N, Campbell G A and Barrett A D T.1997. Antigenic variants of **yellow fever virus** with altered neurovirulence phenotype in mice. Virology 230, 376-380) showing that it encodes an epitope recognized by a wild-type-specific monoclonal. . .

SUMM . . . et al (van der Most R G, Corver J, Strauss J H 1999. Mutagenesis of the RGD motif in the **yellow fever virus** 17D envelope protein. Virology 265, 83-95). It was suggested that the sequence in the RGD loop is critical for the conformation of **E** and minor changes in this region can have drastic effects on the stability of the protein. It is feasible, however, that such changes in structure and/or stability can affect the spectrum of cells infected by influencing the overall **E** protein structure and although that particular area would not be directly involved in the receptor binding. Since this loop is. . .

SUMM . . . Jennings et al (1994) who noted that the 17D virus recovered from a human case of postvaccinal encephalitis had a **E**→K change at position 303 and was found to have increased neurovirulence for both mice and monkeys.

SUMM . . . templates. J. Virol. 66: 5425-5431) allowed the identification

of a mutation (Lys for Glu) at amino acid 136 of the **E** protein which resulted in the loss of neurovirulence for mice (see Sumiyoshi, H.; Tignor, G. H. and Shope, R. **E**. (1996). "Characterization of a highly attenuated Japanese encephalitis virus generated from molecularly cloned cDNA". J. Infect. Dis. 171: 1144-1151). This. . .

SUMM . . . lie outside the structural area, namely, 5' UTR, NS1 and NS3. Little is known about the molecular basis of viscerotropism, i.**e**. the ability of wild type YF virus to replicate and damage extraneural tissue, specially hepatic tissue, or the mutations responsible. . . proteins as well as the 3' end nontranslated region, the latter would presumably restrict replication. Nevertheless, such analyses of the **E** protein provides a framework for understanding several aspects of flavivirus biology and suggests that it should be possible to engineer. . .

SUMM [0020] Being the major component of the virion surface, the envelope protein **E** plays a dominant role in eliciting neutralizing antibodies and the induction of a protective response. This has been conclusively demonstrated by active immunization of animals with defined subviral components and recombinant proteins and by passive protection experiments with **E** protein-specific monoclonal antibodies. Linear epitopes have been mapped using synthetic peptides and are found in areas of the glycoprotein predicted to be hydrophilic, however, the induction of neutralizing antibodies seems to be strongly dependent on the native conformation of **E**. A number of neutralizing sites have been inferred from studies with monoclonal antibody scape mutants and have been mapped onto. . .

SUMM [0021] The neutralization epitopes recognized by monoclonal antibodies are conformational since **E** protein denaturation abolishes binding. Moreover, monoclonal antibodies will only react with synthetic peptides if they recognize an epitope which is present on the denatured **E** protein. Since the dimeric subunit forms part of a as yet undefined lattice on the virion surface, it is likely. . .

SUMM . . . activity and a significant degree of passive protection in mice has been observed by passive immunization with monoclonal antibodies against **prM**. This is due to a certain degree of partial cleavage of **prM** to form M by the furin in the Golgi system and thereby **prM** remains associated with the virus particle being an additional target for antibodies.

SUMM [0026] Vaccination of humans with recombinant poxviruses expressing the structural proteins **prM** and **E** of Japanese encephalitis elicited CD4-CD8+ CTLs directed to the JE virus structural proteins although no specific epitopes were identified (Konish **E**, Kurane I, Mason P W, Shope R **E**, Kanesa-Thasan N, Smucny J J, Hoke C H, Ennis F A 1998. Induction of Japanese-encephalitis virus-specific cytotoxic T lymphocytes in. . .

SUMM [0027] More recently a JE virus **E** protein epitope recognized by JE-specific murine CD8+ CTLs has been reported. The epitope was found to correspond to amino acids 60-68 of the JE virus protein which are located in domain II (Takada K, Masaki H, Konishi **E**, Takahashi M, Kurane I 2000. Definition of an epitope on Japanese encephaltis virus envelope protein recognized by JEV-specific murine CD8+. . .

SUMM [0028] Functional T-helper cell epitopes in the flavivirus **E** protein were identified by measuring B-cell response after immunization with synthetic peptides (Roehrig J T, Johnson A J, Hunt A R 1994. T-helper cell epitopes on the **E** glycoprotein of dengue 2 Jamaica virus. Virology 198, 31-38).

SUMM . . . its residual neurovirulence, as determined by Marchevsky, R. S. et al (Marchevsky R S, Mariano J, Ferreira V S, Almeida **E**, Cerqueira M J, Carvalho R, Pissurno J W, Travassos da Rosa A P A, Simo{tilde over (**e**)}s M C, Santos C N D, Ferreira I I, Muylaert I R, Mann G F, Rice C M and Galler R 1995. Phenotypic analysis of **yellow fever virus** derived from complementary DNA. Am. J. Trop. Med. Hyg. 52, 75-80). Although these results showed the virus was not ideally. . .

SUMM . . . been noted (Galler R, Post P R, Duarte dos Santos C N and Ferreira I I. 1998. Genetic variability among **yellow fever virus** 17D substrains. Vaccine 16, 1024-1028).

SUMM . . . to the antigen to be expressed. One major approach has been the creation of chimeric viruses through the exchange of **prM**/M/**E** genes as first established for DEN-4 virus chimeras (Lai et al, 1998, U.S. Pat. No. 5,494,671). The **prM**/M/**E** genes of dengue virus serotypes 1, 2 and 3 were inserted into the dengue 4 infectious clone resulting in chimeric. . .

SUMM . . . the first chimeric virus developed with the YF 17D cDNA from Rice et al (1989) by the exchange of the **prM**/M/**E** genes with cDNA derived from JE SA14-14-2 and Nakayama strains of JE virus. The former corresponds to the live attenuated. . .

SUMM . . . P, Soike K, Levenbook I, Zhang Z X, Arroyo J, Delagrave S, Myers G, Barrett A D T, Shope R **E**, Rattterree M, Chambers T J, Guirakhoo F 1999. Recombinant, chimeric live, attenuated vaccine

(Chimerivax) incorporating the envelope genes of Japanese. . .

SUMM [0041] Chimeric virus retained nucleotide/amino acid sequences present in the original SA14-14-2 strain. This vaccine strain differs, in **prM**/M/**E** region, from the parental virus in 6 positions (**E**-107; E138; E176: E279; E315; E439). Mutations are stable across multiple passages in cell culture (Vero) and mouse brain but not. . . in FRhL cells. Despite previous data on the genetic stability of such virus, one of the 4 changes in the **E** protein related to viral attenuation had reverted during the passaging to produce the secondary seed.

SUMM [0042] In a dose-response study neutralizing antibodies specific for **prM**/M/**E** were elicited in all groups of monkeys with different doses even with as little as 100 PFUs and conferred full. . .

SUMM . . . 2000. Recombinant chimeric yellow fever-dengue type 2 virus is immunogenic and protective in nonhuman primates. J. Virol. 74, 5477-5485) involved **prM**/M/**E** gene replacement (fusion at the signalase cleavage site) with a den2 cDNA. All virus regeneration and passaging was done in. . . PM cells (a cell bank from Pasteur-Merieux) allegedly certified for live vaccine virus production. Recombinant virus retained the original den2 **prM**/M/**E** sequences even after 18 serial passages in Vero cells but some variation was noted in YF genes. Phenotypic analysis of. . .

SUMM . . . surface of the type 3 virus (Murray M G, Kuhn R J, Arita M, Kawamura N, Nomoto A & Wimmer **E** (1988) Poliovirus type 1/type 3 antigenic hybrid virus constructed in vitro elicits type 1 and type 3 neutralizing antibodies in. . .

SUMM [0050] Bendahmane et al (Bendahmane M, Koo M, Karrer **E**, Beachy R N 1999. Display of epitopes on the suface of tobacco mosaic virus: impact of charge and isoelectric point. . .

SUMM . . . by monoclonal antibody as a neutralizing MVE epitope The chimera, however, was not viable suggesting that particular area of the **E** protein (amino acids 192-193 from the amino terminal) is critical for YF virus viability (R. Weir and C. Rice, pers.. . .

SUMM . . . envelope protein of any Flavivirus, wherein the sites are structurally apart from areas known to interfere with the overall flavivirus **E** protein structure and comprising: sites that lie on the external surface of the virus providing accessibility to antibody; not disrupt or significantly destabilize the three-dimensional structure of the **E** protein and not interfere with the formation of the **E** protein network within the viral envelope.

DRWD [0068] FIG. 2 shows the sequence alignment of the soluble portions of the Envelope proteins from tick-borne encephalitis virus (tbe), **yellow fever virus** (yf), japanese encephalitis virus (je) and Dengue virus type 2 (den2).

DRWD . . . f and g. As in FIG. 2, the alignment shown is that used for model building of the modified yf **E** protein and deliberate misalignments are shown shaded. Elements of secondary structure are shown as horizontal bars between the two sequences.

DRWD [0073] FIG. 7: sets forth two views of the modelled yf **E** protein including the SYVPSAEQI insertion sequence within the fg loop. The domains are coloured individually, domain I (red), domain II (yellow) and domain III (blue). In the upper panel the **E** protein dimer is seen perpendicular to the viral membrane and in the lower panel is viewed within the membrane plane,. . .

DRWD [0074] FIG. 8: sets forth the superposition of ten models of the YF **E** protein including the insertion sequence GG(NANP)$_{3GG}$ within the fg loop. In each model the insertion sequence is shown in a. . .

DRWD [0075] FIG. 9: shows the molecular surface of the YF **E** protein dimer for one of the ten models of FIG. 8. In the upper panel, the blue and red dots. . .

DETD [0086] The fact that the 3-D structure for the flavivirus **E** protein is available (Rey et al, 1995) would support the approach first used for poliovirus by examining the 3D-structure and selecting sites for insertion which are less likely to interfere with the overall **E** protein structure. The major concern about inserting epitopes into flaviviruses **E** protein, in particular into the YF 17D **E** protein, relates to the fact that this protein is the main target for humoral neutralizing response, it is the protein. . . receptor recogniton and cell penetration, therefore targeting the virus to specific cells. By inserting a new epitope somewhere in the **E** protein of a given flavivirus one or more of these properties could be changed unless the analysis of the 3D. . .

DETD [0087] Regarding the Tick-borne encephalitis virus **E** protein, two distinct crystal forms of its soluble fragment were obtained by Rey et al. (Rey et al., 1995). In both, the **E** protein shows a similar dimeric arrangement in which two monomers are related by a molecular twofold axis which is crystallographic. . . in both cases suggests that this is not an artifact of crystallization but represents the true oligomeric arrangement of the **E** protein as inserted into the viral envelope at neutral pH. The dimer presents an elongated flattened structure with

overall dimensions. . .

DETD [0089] On exposure to low pH the network of **E** protein dimers on the viral surface must rearrange into trimers. This must envolve large alterations to the monomer-monomer contacts and. . . G, Holzmann H, Allison S L, Mandl C W, Kunz C 1994 Structural-Changes And Functional Control Of The Tick-Borne Encephalitis-Virus Glycoprotein-**E** By The Heterodimeric Association With Protein **prM** Virology 198, 109-117).

DETD [0090] For the design of insertions of epitopic peptides into the **E** protein of a given Flavivirus and the subsequent evaluation of their viability the inventors of the present invention developed the following strategy. Initially it was necessary to produce a three-dimensional model for the **E** protein of a selected Flavivirus. The sequence of the yellow fever 17DD strain was used for this purpose and its. . . with that of tick-borne encephalitis (tbe) virus was generated initially with the program MULTALIGN (Barton G J, Sternberg M J **E**, 1987, A Strategy For The Rapid Multiple Alignment Of Protein Sequences--Confidence Levels From Tertiary Structure Comparisons J Mol Biol 198,. . .

DETD . . . in tbe leading to a complete readjustment of the alignment up to the region of the glycosylation site between β-strands E_0 and F_0. On comparing the tbe and yellow fever (yf) sequences within the region 120 to 150 (tbe sequence numbering),. . . new alignment accommodates the two residue insertion (122 and 123 in tbe) in a surface loop (between β-strands d and **e**).

DETD [0094] The sequence alignment was used to generate 10 models for the yf **E** protein dimer using satisfaction of spatial restraints derived from the tbe dimeric structure employing the program MODELLER (Sali A, Blundell,. . .

DETD [0096] The model for the yf **E** protein shows a slightly reduced contact area between subunits compared with tbe (1,242 Å^2 per monomer compared with 1,503 Å^2),. . . domain II and the cavity between domains I and III of the opposite subunit is essentially retained in the yf **E** protein.

DETD [0097] The model for the yf **E** protein together with the sequence alignment was used to select potential insertion sites for heterologous B and T cell epitopes. In both cases such an insertion site should 1) not disrupt or significantly destabilize the three-dimensional structure of the **E** protein; 2) not interfere with the formation of the **E** protein network within the viral envelope; 3) lie on the external surface of the virus such that it is accessible. . .

DETD . . . That between loops c and d represents the fusion peptide, is partially buried and highly conserved. That between d and **e** shows little structural variation and includes a 1/2-cystine residue which is structurally important. That between E_0 and F_0 includes the glycosylation site in tbe and is a potential insertion site as it shows great structural variability. . . between B and C_x is a possible site but may form part of the lateral surface. That between D_x and **E** shows structural variation and presents an asparagine-rich sequence in yf which may accommodate asparagine-rich epitopes. From the above, the most. . . dimer interface for a large insertion without creating steric hindrance. Besides the fg loop another promising insertion site is the E_{0F0} as it shows great structural variability and is highly exposed. Although not wishing to be bound by any particular theory,. . .

DETD . . . and expression of particular antigens, including epitopes, into sites structurally apart from areas known to interfere with the overall flavivirus **E** protein structure, specially into the fg loop or the E_{0F0} loop of a given flavivirus **E** protein. The foreign inserted antigen, including epitope, may vary widely dependent on the immunogenic properties desired in the antigen. For. . .

DETD [0100] More particularly, one strategy described here is the insertion of malarial gene sequences into the fg loop of YF17D **E** protein. While comparatively short sequences having only a few amino acid residues may be inserted, it is also contemplated that. . . of the antigen/epitope will depend on the fact that it would not compromise the structure and the function of the **yellow fever virus** envelope.

DETD . . . initiate the infection of red blood cells. Antigens specific for the liver stage have been identified (Calle J M, Nardin **E** H, Clavijo P, Boudin C, Stuber D, Takacs B, Nussenzweig R S & Cochrane A H. 1992. Recognition of different. . . Plasmodium falciparum CS protein by the sera of naturally infected individuals compared with those of sporozoite-immunized volunteers.J Immunol. 49(8):2695-701; Nardin, **E** H & Nussenzweig, R S.1993.T cell responses to pre-erythrocytic stages of malaria: role in protection and vaccine development against pre-erythrocytic. . .

DETD . . . in immunity to asexual blood stages. Potentially these antibodies could neutralize parasites or lead to Fc-dependent mechanisms of parasite killing, **e**.g., macrophages. Complete protection against sporozoite challenge observed in irradiated P. berghei

sporozoite-immunized mice and P.falciparum sporozoite-inmunized humans results from immune responses to antigens expressed by the parasite at the preerythrocytic stages of its life cycle (Nardin **E** H, Nussenzweig R S 1993. T cell responses to pre-erythrocytic stages of malaria: role in protection and vaccine development against. . .

DETD . . . plasmodial proteins are being expressed in different systems towards immunogenicity studies (Munesinghe D Y, Clavijo P, Calle M C, Nardin **E** H, Nussenzweig R S 1991. Immunogenicity of multiple antigen peptides (MAP) containing T and B cell epitopes of the repeat region of the P.falciparum circunsporozoite protein. Eur.J.Immunol. 21, 3015-3020; Rodrigues et al 1994; Shi Y A, Hasnain S **E**, Sacci J B, Holloway B P, Fujioka H, Kumar N, Wohlhueter R, Hoffman S L, Collins W **E**, Lal A A 1999. Immunogenicity and in vitro efficacy of a recombinant multistage Plasmodium falciparum candidate vaccine. Proc. Natl. Acad.. . .

DETD [0107] FIG. 3 shows a schematic representation of the CS protein of Plasmodium sp. (Nardin **e** Nussenzweig, 1993) and the location of epitopes expressed by recombinant YF 17D viruses of the present invention. The CS protein. . .

DETD . . . although there was a significant delay on the onset of parasitemia (parasitemia (Ockenhouse C F, Sun P F, Lanar D **E**, Wellde B T, Hall B T, Kester K, Stoute J A, Magill A, Krzych U, Farley L, Wirtz R A,. . .

DETD . . . response. This immunization also protected mice against infection by sporozoites (Tsuji M, Bergmann C C, Takita-Sonoda Y, Murata K, Rodrigues **E** G, Nussenzweig R S, Zavala F 1998. Recombinant Sindbis virus expressing a cytotoxic T-lymphocyte epitope of a malaria parasite or. . .

DETD . . . the CS protein of P.yoelii elicits a high degree of resistance to infection mediated primarily by CD8+ T cells (Rodrigues **E** G, Zavala F, Eichinger D, Wilson J M, Tsuji M 1997. Single immunizing doses of recombinant adenovirus efficiently induces CD8+. . .

DETD . . . and express single defined antigens, including epitopes into sites structurally apart from areas known to interfere with the overall flavivirus **E** protein structure, specially into the fg loop or the $\mathbf{E}_{0F0}$ loop of the **E** protein of a given flavivirus, such as yellow fever, dengue, Japanese encephalitis, tick-borne encephalitis, that can be used as new. . . the present invention is related to a general approach to express single defined epitope on the fg loop of the **E** protein of a YF 17D virus.

DETD . . . virus. The phenotype of this virus has been tested in monkeys (Marchevsky R S, Mariano J, Ferreira V S, Almeida **E**, Cerqueira M J, Carvalho R, Pissurno J W, Travassos da Rosa A P A, Simoes M C, Santos C N. . . D, Ferreira I I, Muylaert I R, Mann G F, Rice C M and Galler R. 1995. Phenotypic analysis of **yellow fever virus** derived from complementary DNA. Am. J. Trop. Med. Hyg. 52, 75-80) and these studies indicated the need for genetic modification. . .

DETD . . . replication origin that allows only limited replication of the plasmid reducing the number of plasmid DNA molecules per bacterial cells, i.**e**. vectors consisting of low copy number plasmids such as pBeloBAC11 (Almazan F, Gonzalez J M, Penzes Z, Izeta A, Calvo **E**, Plana-Duran J, Enjuanes L. 2000 Engineering the largest RNA virus genome as an infectious bacterial artificial chromosome.ProcNatlAcadSciUSA97:5516-5521). Another possibility is. . .

DETD . . . restriction sites of pACNR1180 by digestion with NdeI/SalI, filling in the ends by treating with Klenow enzyme, ligating and transforming **E**.coli XL1-blue.

DETD . . . the opposite strand as the complete genome spans nucleotide 12,817 to 1951. All insertions at the fg loop of the **yellow fever virus E** protein are made at the EcoRV site of YFE200 plasmid and from there incorporated into pYF17D/14 by exchanging fragments NsiI/NotI.. . .

DETD . . . the insertion of foreign sequences into the fg loop as defined by the analysis of the 3D structure of the **E** protein we have created by in vitro mutagenesis a restriction site (EcoRV) at nucleotide 1568. The creation of this site led to two amino acid changes in the **E** protein at positions **E**-199 and **E**-200 (**E**→D, T→I, respectively).

DETD . . . of chimeric 17D/JE-Nakayama in the mouse model of encephalitis (Chambers et al, 1999). In addition no major alterations in the **E** protein structure was apparent when modelling these amino acids changes into the predicted 3D model. Finally, the YF17D/G1/2-derived virus grew. . .

DETD . . . Freire (U.S. Pat. No. 6,171,854) and herein. It contains 6905 nucleotides and region 1-2271 corresponds to the 5' UTR, C, **prM**/M and **E** genes. This region is fused through an EcoRI site at the **E** gene (2271) to another EcoRI site in the NS5 gene (position 8276). At position 1568 in the **E** gene we created the EcoRV site which is used for epitope insertion into the **E** protein fg loop. This plasmid also consists of the NS5 gene from nucleotide 8276 to the last YF genome

nucleotide. . .

DETD [0139] Using an identical approach to that described above the insertion DYENDIEKKI was introduced into the yf **E** protein. An identical alignment to that shown in FIG. 6 was used in this case with the exception of the. . .

DETD [0141] Compared with the loop observed experimentally in the crystal structure of the **E** protein from the the virus, the two insertions described above are three residues longer. These additional residues are accommodated in. . .

DETD . . . probability that that which is immunologically relevant may be adopted, even within the context of its insertion into the yf **E** protein. FIG. 9 illustrates better the volume considerations.

DETD . . . that the envelope proteins of japanese encephalitis virus and Dengue 2 would accommodate the above described insertions equally well as **yellow fever virus**. The alignment of FIG. 2 shows that a similar six-residue deletion is present in all three viral envelope proteins compared to tbe. Models for the je and d2 **E** protein, produced using a similar protocol to that for yf, frequently show a β-turn structure for the fg loop, stabilized. . . contacts to those of yf are also observed around the distal dimer interface site. A representative model for the je **E** protein has a PROCHECK G-factor of -0.1, 89.9% of residues in the most favourable regions of the Ramachandran plot, good. . .

DETD [0147] Additionally, the site which comprises the region of E_0 and F_0 strands including the E_{0F0} loop which form part of the eight stranded β-barrel of domain I of the flavivirus envelope protein comprises the region. . . reference to the tick-borne encephalitis virus sequence described in FIG. 2. More particularly, the site is the loop area between E_0 and F_0 strands which form part of the eight stranded β-barrel of domain I (amino acid 146 to 160 with. . .

DETD . . . restriction sites of pACNR1180 by digestion with NdeI/SalI, filling in the ends by treating with Klenow enzyme, ligating and transforming **E**.coli XL1-blue. This new version of pACNR1180 was named pACNR1180Nde/Sal.

DETD . . . opposite strand as the complete genome spans nucleotide 12,817 to 1951. All insertions at the fg loop of 17D virus **E** protein are made at the EcoRV site of YFE200 plasmid and from there incorporated into pYF17D/14 by exchanging fragments NsiI/NotI.. . .

DETD [0156] To prepare plasmids DNAs from bacteria, glycerol stocks of the **E**. coli harboring each of the two YF plasmids, pYFE200 and pYF17D/14 must be available. Luria Broth-50% glycerol media is used. . .

DETD . . . the cell culture supernatant derived from the transfection.

TABLE 1

Amino acid sequence and specificity of $(NANP)_3$ humoral epitope for insertion into YF **E** protein

Sequence	Antigen epitope	source	Clone
EMD GGNANPNANPNANPGG IES	CSP-B	P.falciparum	17D/8

DETD [0170] To investigate the expression of the 16-amino acid epitope in the **E** protein of 17D/8 virus it was performed an indirect immunofluorescence assay using a monoclonal antibody directed to the $(NANP)_3$ repeat ((MAb 2A10; Zavala F, Cochrane A H, Nardin **E** H, Nussenzweig R S, Nussenzweig V 1983. Circunsporozoite proteins of malaria parasites contain a single immunodominant region with two or. . .

DETD . . . 17D/14 (lanes 4,5,6) or 17D/8 (lanes 7,8,9) virus-infected monolayers. These different extracts were immunoprecipitated with a murine hyperimmune serum against **yellow fever virus** from ATCC (lanes 1,4,7), $(NANP)_3$ repeat-specific monoclonal antibody or 2A10 (lanes 2,5,8) and with two monoclonal antibodies directed against NS1. . .

DETD . . . to YF 17D NS1 protein (FIG. 11 lanes lanes 4, 6, 7 and 9). However, Mab 2.10 precipitated exclusively the **E** protein of the recombinant 1 7D/8 virus (lanes 5 and 8) consistent with the interpretation of correct expression and exposure. . .

DETD [0176] A third set of experiments to show the correct **E** protein surface expression of the $(NANP)_3$ epitope was to examine viral neutralization by specific sera. We used a plaque reduction. . .

DETD . . . recognition is not hindered by its involvement in other viral epitope structures. It is also the first demonstration that a **E** protein linear epitope can be neutralizing for a flavivirus.

DETD . . . RNA was collected and subjected to nucleotide sequence determination (as RT-PCR products) at and around the insertion site in

the **E** protein.
DETD . . . (10b) -
Vero

17D/8 (5 c)	+	17D/8 (10 c)	+
17D/8 (5 d)	+	17D/8 (10 d)	+
17D/8 (5 **e**)	+	17D/8 (10 **e**)	+
17D/8 (5 f)	+	17D/8 (10 f)	+
17D/8 (5 g)	+	17D/8 (10 g)	+

*The letters a and b represent two independent passages in CEF cells, whereas c, d, **e**, f, and g the five independent series of passages in Vero cells.
DETD . . . to other viruses in Table 6.
TABLE 6

Comparison of YF infectious plasmid clone sequences.

NT/gene	YFiv5.2[a]	17D/DD[b]	YFiv5.2/DD[c]	17D/8	17D/1	17D/13	NTAA
1140/**E**	T	C	C	T	T	T	TVal→CAla
1436,1437/**E**	G,A	A,G	A,G	G,A	G,A	G,A	G,AD→A,GS
1946/**E**	T	C	C	T	C	C	TS→CP
2219,2220/**E**	A,C	G,T	G,T	AC	G,T	G,T	A,CT→G,TV
2356/**E**	T	T	C	T	T	T	--
2602/NS1	T	T	C	T	T	T	--
2677/NS1	C	C	T	C	C	C. . .	

DETD . . . suggested that the epitope is being presented in the correct conformation and is accessible to antibodies either in solubilized native **E** protein as well as on the virus surface. In this example it was looked further into the immunogenicity of the. . .
DETD . . . shows the predicted charge and isoelectric points for the epitopes alone, integrated into the fg loop and in the whole **E** protein context. As can be seen there is considerable variation of the net charge and the pI in each context, epitope alone, in the loop or in whole **E** contexts. Since the insertion region is involved in the pH-dependent conformatinal transition for fusion of the envelope to endosome membrane. . .
DETD . . . Vero cells (FIG. 13). Table 8 shows the amino acid sequence and specificity of selected epitopes for insertion into YF **E** protein.
TABLE 8

Amino acid sequence and specificity of selected epitopes for insertion into YF **E** protein.

EMD	GGNANPNANPNANPGG	YF fg loop	DYENDIEKKI IES	EMD IES	EMD SYVPSAEQI IES
Antigen epitope			CSP-CTL	CSP-B*	CSP-CTL**
Source			P.falciparum	P.falciparum	P.yoelii
Clone			17D/1	17D/8	17D/13
Charge/Peptide pI[a]	--	-2.00/4.06		0.00/5.97	-1.01/3.30
fg loop + epitope charge/pI[a]	-2.00/3.69	-4.99/3.83		-4.00/3.49	-3.00/3.58
YF **E** protein + epitope charge/pI[a]	-7.4/5.83	-9.40/5.61		-7.40/5.83	-8.40/5.71

*B, B cell
**CTL, cytotoxic T cell epitope
DETD [0212] There are no significant differences in the overall charge and isoelectric point of the whole **E** protein containing these epitopes but differences can be observed at the level of the fg loop (see Table 8) containing. . .
DETD . . . RNA was collected and subjected to nucleotide sequence determination (as RT-PCR products) at and around the insertion site in the **E** protein.
DETD [0225] Epitope insertion at this site may affect the threshold of

fusion-activated conformational change of the **E** protein and it is conceivable that a slower rate of fusion may delay the extent of virus production and thereby. . .

DETD . . . suggests that also in nonhuman primates the recombinant virus has maintained its immunogenicity despite the epitope insertion in its envelope **E** protein.

DETD . . . vaccine counterpart. The testing of one such recombinant virus (17D/13) for monkey neurovirulence also suggested that insertion at the 17D **E** protein fg loop does not compromise its atttenuated phenotype further confirming the potential use of this site for the insertion. . .

DETD [0256] I.e) molecule type: cDNA

CLM What is claimed:

. . . envelope protein of any Flavivirus, wherein the sites are structurally apart from areas known to interfere with the overall flavivirus **E** protein structure and comprising: (i) sites that lie on the external surface of the virus providing accessibility to antibody; (ii) not disrupt or significantly destabilize the three-dimensional structure of the **E** protein; and, (iii) not interfere with the formation of the **E** protein network within the viral envelope.

6. The method according to claim 1 wherein another site comprises the region of E_0 and F_0 strands including the E_{0F0} loop which form part of the eight stranded β-barrel of domain I.

7. The method according to claim 6 wherein the site is the loop area between E_0 and F_0 strands which form part of the eight stranded β-barrel of domain I.

. . . 10. The method according to claim 1 wherein the Flavivirus is selected from the group consisting of any Flavivirus including **yellow fever virus**, tick borne encephalitis virus, dengue virus and japanese encephalitis virus.

12. The method according to claims 10 and 11 wherein the Flavivirus is a **yellow fever virus**.

13. The method according to claim 12 wherein the virus is a recombinant **yellow fever virus**.

14. The method according to claim 13 wherein the **yellow fever virus** is the YF17D virus strain and substrains thereof.

. . . The DNA construct according to claim 20 wherein the Flavivirus is selected from the group consisting of any Flavivirus including **yellow fever virus**, tick borne encephalitis virus, dengue virus and japanese encephalitis virus

. . . level of its envelope protein, wherein the sites are structurally apart from areas known to interfere with the overall flavivirus **E** protein structure.

42. The Flavivirus according to claim 37 wherein another site comprises the region of E_0 and F_0 strands including the E_{0F0} loop which form part of the eight stranded β-barrel of domain I.

43. The Flavivirus according to claim 42 wherein the site is the loop area between E_0 and F_0 strands which form part of the eight stranded β-barrel of domain I.

. . . 46. The Flavivirus according to claim 37 wherein the Flavivirus is selected from the group consisting of any Flavivirus including **yellow fever virus**, tick borne encephalitis virus, dengue virus and japanese encephalitis virus.

48. The Flavivirus according to claims 46 and 47 wherein the Flavivirus is a **yellow fever virus**.

49. The Flavivirus according to claim 48 wherein the virus is a recombinant **yellow fever virus**.

50. The Flavivirus according to claim 49 wherein the **yellow fever virus** is the YF17D virus strain and substrains thereof.

53. The vaccine composition according to claim 52 wherein the flavivirus is a **yellow fever virus** and the other infectious agent is the causative agent of malaria.

L6 ANSWER 2 OF 7 USPATFULL on STN
2003:257279 Viral vaccine production method.
Monath, Thomas P., Harvard, MA, UNITED STATES
Guirakhoo, Farshad, Melrose, MA, UNITED STATES
Arroyo, Juan, S. Weymouth, MA, UNITED STATES
US 2003180329 A1 20030925
APPLICATION: US 2003-342681 A1 20030115 (10)
PRIORITY: US 2002-348565P 20020115 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

SUMM [0008] Fully processed, mature virions of flaviviruses contain three structural proteins, envelope (**E**), capsid (C), and membrane (M), and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Immature flavivirions found in infected cells contain pre-membrane (**prM**) protein, which is the precursor to the M protein. The flavivirus proteins are produced by translation of a single, long. . . Virology, Fields (ed.), Raven-Lippincott, New York, 1995, Volume I, p. 937). The virus structural proteins are arranged in the order C-**prM-E**. These proteins are present in the N-terminal region of the polyprotein, while the non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) are located in the C-terminal region of the polyprotein. The amino termini of **prM**, **E**, NS1, and NS4B are generated by host signalase cleavage within the lumen of the endoplasmic reticulum (ER), while most cleavages. . .

SUMM . . . against flavivirus infection has involved the construction of live, attenuated chimeric flaviviruses. In one example of such a chimera, the **prM** and **E** proteins of one flavivirus (**e**.g., **yellow fever virus**) are replaced by the **prM** and **E** proteins of another flavivirus (**e**.g., Japanese encephalitis, dengue, or West Nile virus), to which immunity is sought. Details of the construction and use of such. . .

SUMM [0010] The invention provides methods of producing vaccines containing live, attenuated viruses (**e**.g., flaviviruses, such as a **yellow fever virus**, or chimeric viruses). These methods involve (i) introducing a nucleic acid molecule corresponding to the genome of the virus into heteroploid cells (**e**.g., Vero cells); (ii) treating virus harvested from the cells with a nuclease (**e**.g., Benzoase®); and (iii) formulating the nuclease-treated virus for administration as a vaccine. Further, the methods can, optionally, include the step. . .

SUMM . . . the virus produced using these methods is a chimeric flavivirus. Such a chimeric flavivirus may include, for example, (i) a **yellow fever virus** in which the nucleotide sequence encoding a **prM-E** protein is either deleted, truncated, or mutated so that functional **yellow fever virus prM-E** protein is not expressed, and (ii) integrated into the genome of the **yellow fever virus**, a nucleotide sequence encoding a **prM-E** protein of a second, different flavivirus, so that the **prM-E** protein of the second flavivirus is expressed. The second flavivirus may be, for example, Japanese Encephalitis virus, a Dengue virus (**e**.g., one of Dengue types 1, 2, 3, and 4), a Murray Valley Encephalitis virus, a St. Louis Encephalitis virus, a West Nile virus, a Tick-borne Encephalitis virus (**e**.g., Central European Encephalitis virus or Russian Spring-Summer Encephalitis virus), a Hepatitis C virus, a Kunjin virus, a Powassan virus, a. . .

SUMM [0012] In the chimeric viruses used in the invention, the nucleotide sequence encoding the **prM-E** protein of the second, different flavivirus preferably replaces the nucleotide sequence encoding the **prM-E** protein of the **yellow fever virus**. Also, the **prM** signal of the chimeric virus is, preferably, that of **yellow fever virus**.

SUMM . . . the large-scale production of viral vaccines, as they can be grown on beads, and thus can be produced in large (**e**.g., 1,000-2,000 L) biofermentors. In addition, these cells do not produce interferon, which inhibits viral replication. Further, the number of purification. . .

DETD . . . producing vaccines that contain live, attenuated viruses. These methods can be used, for example, in the production of flavivirus vaccines (**e**.g., chimeric flavivirus vaccines). In general, the methods of the invention involve the introduction of a nucleic acid molecule (**e**.g., an RNA molecule) corresponding to the genome of a virus into heteroploid cells (**e**.g., Vero cells), harvesting virus from the medium in which the cells have been cultured, treating virus obtained in this manner with a nuclease (**e**.g., an endonuclease that degrades both DNA and RNA, such as Benzonase.TM.; U.S. Pat. No. 5,173,418), concentrating the nuclease-treated virus (**e**.g., by use of ultrafiltration using a filter having a molecular weight cut-off of, **e**.g., 500 kDa), and formulating the concentrated virus for the purposes of vaccination. Details of the methods of the invention are. . .

DETD . . . type of live, attenuated virus. Preferably, the virus is a live, attenuated flavivirus. For example, vaccine containing a live, attenuated **yellow fever virus** (**e**.g., YF17D) can be produced. Other examples of viruses that can be included in vaccines produced using the methods of the. . .

DETD . . . can be included in the vaccines that are made using the methods of the invention can consist of a flavivirus (i.**e**., a backbone flavivirus) in which a structural protein (or proteins) has been replaced with a corresponding structural protein (or proteins) of a second virus (i.**e**., a test or a predetermined virus, such as a flavivirus). For example, the chimeras can consist of a backbone flavivirus in which the **prM** and **E** proteins of the flavivirus have been replaced with the **prM** and **E** proteins of the second, test virus.

DETD . . . Hemorrhagic fever, Louping ill, Powassan, Negishi, Absettarov, Hansalova, Apoi, and Hypr viruses; as well as viruses from the Hepacivirus genus (**e**.g., Hepatitis C virus). Additional viruses that can be used as the source of inserted structural proteins include viruses from the Pestivirus genus (**e**.g., Bovine diarrhea virus), and other viruses, such as Lassa, Ebola, and Marburg viruses.

DETD . . . the vaccines produced using the methods of the invention is the yellow fever human vaccine strain, YF17D, in which the **prM** and **E** proteins have been replaced with **prM** and **E** proteins of another flavivirus, such as Japanese encephalitis virus, West Nile virus, St. Louis encephalitis virus, Murray Valley encephalitis virus,. . .

DETD [0033] A full-length cDNA template of yellow fever 17D in which the genes encoding the structural proteins (**prM** and **E**) YF 17D are replaced with the corresponding genes of an attenuated JE SA14-14-2 virus was used as a template for RNA synthesis. Full-length RNA was then synthesized using SP6 polymerase. Since flaviviruses have positive-sense RNA (i.**e**., they are infectious and serve as message for translation of all of the proteins required for replication), the chimeric RNA. . .

DETD . . . virus. The vaccine candidate ChimeriVax.TM.-JE is a live, attenuated, genetically-engineered virus prepared by replacing the genes encoding two structural proteins (**prM** and **E**) of yellow fever 17D vaccine virus with the corresponding genes of an attenuated strain of JE virus (Chambers et al., 1998). The **prM** and **E** proteins of JE virus contain the critical antigens conferring protective humoral and cellular immunity, as shown by many previous studies. . .

DETD [0041] The JE **prM** and **E** genes in the chimeric vaccine candidate were derived from the JE SA14-14-2 strain (a live, attenuated vaccine strain licensed for. . . well documented. The genetic rearrangement was accomplished by standard cloning techniques, employing two bacterial plasmids containing cDNA copies of the **prM-E** genes of JE SA14-14-2 virus and the remaining genes of yellow fever 17D. A full-length `chimeric` yellow fever-JE infectious cDNA. . .

DETD . . . sequences within the YF5'3'IV JE PrME and YFM5.2 JE plasmids by the corresponding JE sequences from the start of the **prM** protein (nt 478, amino acid (aa) 128) through the **E**/NS1 cleavage site (nt 2452, aa 817) (Chambers et al., J. Virol. 73(4):3095-3101, 1999; Rice et al., New Biol. 1:285-296, 1989).. . .

DETD . . . art, and can readily be adapted for use in the present invention by those of skill in this art. (See, **e**.g., Remington's Pharmaceutical Sciences (18th edition), ed. A. Gennaro, 1990, Mack Publishing Co., Easton, Pa.) However, the viruses can simply be. . .

DETD . . . be used to enhance the immunogenicity of the chimeric vaccines include, for example, liposomal formulations, synthetic adjuvants, such as saponins (**e**.g., QS21), muramyl dipeptide, monophosphoryl lipid A, or polyphosphazine. Although these adjuvants are typically used to enhance immune responses to inactivated. . .

DETD . . . For example, the live, attenuated virus can be formulated as sterile aqueous solutions containing between 100 and 1,000,000 infectious units (**e**.g., plaque-forming units or tissue culture infectious doses) in a dose volume of 0.1 to 1.0 ml, to be administered by,. . .

CLM What is claimed is:

3. The method of claim 2, wherein said flavivirus is a **yellow fever virus**.

5. The method of claim 4, wherein said chimeric flavivirus comprises a **yellow fever virus** in which the nucleotide sequence encoding a **prM-E** protein is either deleted, truncated, or mutated so that functional **yellow fever virus prM-E** protein is not expressed, and integrated into the genome of said **yellow fever virus**, a nucleotide sequence encoding a **prM-E** protein of a second, different flavivirus, so that said **prM-E** protein of said second flavivirus is expressed.

10. The method of claim 5, wherein the nucleotide sequence encoding the **prM-E** protein of said second, different flavivirus replaces the

nucleotide sequence encoding the **prM-E** protein of said **yellow fever virus**.

11. The method of claim 5, wherein the **prM** signal of said chimeric virus is that of **yellow fever virus**.

L6 ANSWER 3 OF 7 USPATFULL on STN
2003:64652 Chimeric flavivirus vectors.
Kleanthous, Harold, Westford, MA, UNITED STATES
Miller, Charles, Medford, MA, UNITED STATES
Oros, Larisa, Boston, MA, UNITED STATES
US 2003044773 A1 20030306
APPLICATION: US 2002-160939 A1 20020531 (10)
PRIORITY: US 2001-295265P 20010601 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

SUMM . . . Raven-Lippincott, New York, 1995, Volume 1, p. 937). The virus structural proteins are arranged in the polyprotein in the order C-**prM-E**, where "C" is capsid, "**prM**" is a precursor of the viral envelope-bound M protein, and "**E**" is the envelope protein. These proteins are present in the N-terminal region of the polyprotein, while the non-structural proteins (NS1,. . .

SUMM [0004] A chimeric flavivirus that includes the C and non-structural proteins of the **Yellow fever virus** vaccine strain (YF 17D) and the **prM** and **E** proteins of a strain of attenuated Japanese encephalitis virus (SA 14-14-2) has been made. This chimera, designated ChimeriVax.TM.-JE, has been. . .

SUMM [0005] A similar chimera was made that includes the C and non-structural proteins of YF 17D and the **prM** and **E** proteins of a Dengue-2 strain. This chimera, designated ChimeriVax-D2, was shown to induce neutralizing antibodies against Dengue-2 virus in rhesus. . .

SUMM . . . can be chimeric flavivirus vectors that include, for example, the C and non-structural proteins of a first flavivirus and the **prM** and **E** proteins of a second flavivirus. The first and second flaviviruses can be selected from the group consisting of Japanese encephalitis, Dengue (serotype 1, 2, 3, or 4), Yellow fever (**e**.g., YF 17D), Murray Valley encephalitis, St. Louis encephalitis, West Nile, Kunjin, Rocio encephalitis, Ilheus, ticke-borne encephalitis, Central European encephalitis, Siberian. . .

SUMM . . . includes flavivirus vectors that include envelope proteins that contain foreign peptides. The flavivirus vectors can be chimeric flaviviruses including the **prM** and **E** proteins of a first flavivirus and the C and non-structural proteins of a second flavivirus. The first and second flaviviruses can be selected from the group consisting of Japanese encephalitis, Dengue (serotype 1, 2, 3, or 4), Yellow fever (**e**.g., YF 17D), Murray Valley encephalitis, St. Louis encephalitis, West Nile, Kunjin, Rocio encephalitis, Ilheus, ticke-borne encephalitis, Central European encephalitis, Siberian. . .

DETD [0028] The invention provides methods of identifying sites in the envelope proteins of chimeric flaviviruses or genetically attenuated flaviviruses (**e**.g., YF 17D) into which foreign peptides can be introduced, chimeric flavivirus vectors having envelope proteins that include such peptides, and. . .

DETD [0030] Chimeric viruses that can be used in the invention consist of a first flavivirus (i.**e**., a backbone flavivirus) in which a structural protein (or proteins) has been replaced with a corresponding structural protein (or proteins) of a second virus. For example, the chimeras can consist of a first flavivirus in which the **prM** and **E** proteins have been replaced with the **prM** and **E** proteins of a second virus.

DETD . . . Hemorrhagic fever, Louping ill, Powassan, Negishi, Absettarov, Hansalova, Apoi, and Hypr viruses; as well as viruses from the Hepacivirus genus (**e**.g., Hepatitis C virus).

DETD . . . virus that can be used in the invention is the yellow fever human vaccine strain, YF 17D, in which the **prM** and **E** proteins have been replaced with **prM** and **E** proteins of another flavivirus, such as Japanese encephalitis virus, West Nile virus, St. Louis encephalitis virus, Murray Valley encephalitis virus,. . .

DETD . . . can each include a single epitope. Alternatively, multiple epitopes can be inserted into the vectors, either at a single site (i.**e**., as a polytope, in which the different epitopes can be separated by a flexible linker, such as a polyglycine stretch. . .

DETD . . . is noted in Table 4, epitopes that are used in the vectors of the invention can be B cell epitopes (i.**e**., neutralizing epitopes) or T cell epitopes (i.**e**., T helper and cytotoxic T cell-specific epitopes).

DETD . . . invention can include ligands that are used to target the vectors to deliver peptides, such as antigens, to particular cells (**e**.g., cells that include receptors for the ligands) in subjects to whom the vectors administered.

DETD . . . art. The vectors can be administered and formulated, for example, in the same manner as the yellow fever 17D vaccine, **e**.g., as a clarified suspension of infected chicken embryo tissue, or a fluid harvested from cell cultures infected with the chimeric **yellow fever virus**. Thus, the vectors of the invention can be formulated as sterile aqueous solutions containing between 100 and 1,000,000 infectious units (**e**.g., plaque-forming units or tissue culture infectious doses) in a dose volume of 0.1 to 1.0 ml, to be administered by,. . .

DETD . . . can be used to enhance the immunogenicity of the chimeric vectors include, for example, liposomal formulations, synthetic adjuvants, such as (**e**.g., QS21), muramyl dipeptide, monophosphoryl lipid A, or polyphosphazine. Although these adjuvants are typically used to enhance immune responses to inactivated. . . of a chimeric vector delivered via a mucosal route, for example, orally, mucosal adjuvants such as the heat-labile toxin of **E**. coli (LT) or mutant derivations of LT can be used as adjuvants. In addition, genes encoding cytokines that have adjuvant. . .

DETD . . . envelope gene of ChimeriVax.TM.-JE that are permissive to foreign DNA. As is discussed in further detail below, random mutagenesis in **E**. coli of the gene encoding the JE envelope protein with EZ::TN identified a bank of stable insertion mutants that carried. . .

DETD [0056] Insertion mutants were identified in **E**. coli by selection on LB agar plus kanamycin (50 μg/ml). PCR with TN1.F/TN2.R on select kanamycin-resistant clones following transposition revealed. . .

DETD [0060] The antibiotic resistance marker was removed from stable **E**. coli clones, which were then re-ligated, leaving a 57 basepair in-frame insertion that included a 9-basepair target site sequence duplication. . .

DETD [0067]

TABLE 2

List of examples of pathogens from which antigens/peptides can be derived

	VIRUSES:
	Flaviviridae
	Yellow Fever virus
	Japanese Encephalitis virus
	Dengue virus, types 1, 2, 3 & 4
	West Nile Virus
	Tick Borne Encephalitis virus
	Hepatitis C virus (**e**.g., genotypes 1a, 1b, 2a, 2b, 2c, 3a, 4a, 4b, 4c, and 4d)
	Papoviridae:
	Papillomavirus
	Retroviridae
	Human Immunodeficiency virus, type I
. . .	Mumps virus
	Orthomyxoviridae
	Influenza virus
	Filoviridae
	Marburg virus
	Ebola virus
	Rotoviridae:
	Rotavirus
	Coronaviridae
	Coronavirus
	Adenoviridae
	Adenovirus
	Rhabdoviridae
	Rabiesvirus
	BACTERIA:
	Enterotoxigenic **E**. coli
	Enteropathogenic **E**. coli
	Campylobacter jejuni
	Helicobacter pylori
	Salmonella typhi
	Vibrio cholerae
	Clostridium difficile
	Clostridium tetani
	Streptococccus pyogenes
	Bordetella pertussis
	Neisseria meningitides
	Neisseria. . .

DETD [0068]

TABLE 3

Examples of select antigens from listed viruses

VIRUS	ANTIGEN
Flaviviridae	
Yellow Fever virus	Nucleocapsid, M & **E** glycoproteins
Japanese Encephalitis virus	Nucleocapsid, M & **E** glycoproteins
Dengue virus, types 1, 2, 3 & 4	Nucleocapsid, M & **E** glycoproteins
West Nile Virus	Nucleocapsid, M & **E** glycoproteins
Tick Borne Encephalitis virus	Nucleocapsid, M & **E** glycoproteins
Hepatitis C virus	Nucleocapsid, E1 & E2 glycoproteins
Papoviridae:	
Papillomavirus E6	L1 & L2 capsid protein, & E7 transforming. .

.

CLM What is claimed is:

6. The method of claim 5, wherein said second flavivirus is **Yellow Fever virus**.

14. The flavivirus vector of claim 13, wherein said vector is a chimeric flavivirus comprising the **prM** and **E** proteins of a first flavivirus and the C and non-structural proteins of a second flavivirus.

18. The flavivirus vector of claim 17, wherein said second flavivirus is **Yellow Fever virus**.

L6 ANSWER 4 OF 7 USPATFULL on STN

2003:30900 Nucleic acid vaccines for prevention of flavivirus infection.

Chang, Gwong-Jen J., Fort Collins, CO, UNITED STATES

US 2003022849 A1 20030130

APPLICATION: US 2001-826115 A1 20010404 (9)

PRIORITY: US 1998-87908P 19980604 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

SUMM [0004] The flaviviruses contain the following three structural proteins: **prM**/M, the premembrane and membrane protein; **E**, the envelope protein; and C, the capsid protein. (Monath, in Virology (Fields, ed.), Raven Press, New York, 1990, pp. 763-814;. . . and Neurath, eds.), Elsevier, Amsterdam, 1990, pp. 289-305). M has a molecular weight (MW) of about 7-8 kilodaltons (kDa) and **E** has a MW of about 55-60 kDa. M is synthesized as a larger precursor termed **prM**. The pr portion of **prM** is removed when **prM** is processed to form M protein in mature virions. M and **E** are located in the membrane of the flavivirus particle, and so have long been considered to constitute important immunogenic components. . .

SUMM . . . West Nile virus (Lanciotti et al., Science 286: 2331-2333 (1999)), Powassan virus (Mandl et al., Virology 194: 173-184 (1993)) and **yellow fever virus** (YFV) (Rice et al., Science 229: 726-733 (1985)).

SUMM . . . Such culture methods are cumbersome and expensive. Furthermore, there is the attendant risk of incorporating antigens from the host cells, i.**e**., the brain or other host, into the final vaccine product, potentially leading to unintended and undesired allergic responses in the. . .

SUMM . . . recombinant subunit and viral vaccines have been devised in recent years. U.S. Pat. No. 4,810,492 describes the production of the **E** glycoprotein of JEV for use as the antigen in a vaccine. The corresponding DNA is cloned into an expression system in order to express the antigen protein in a suitable host cell such as **E**. Coli, yeast, or a higher organism cell culture. U.S. Pat. No. 5,229,293 discloses recombinant baculovirus harboring the gene for JEV **E** protein. The virus is used to infect insect cells in culture such that the **E** protein is produced and recovered for use as a vaccine.

SUMM [0018] U.S. Pat. No. 5,021,347 discloses a recombinant vaccinia virus genome into which the gene for JEV **E** protein has been incorporated. The live recombinant vaccinia virus is used as the vaccine to immunize against JEV. Recombinant vaccinia viruses and baculoviruses in which the viruses incorporate a gene for a C-terminal truncation of the **E** protein of dengue serotype 2, dengue serotype 4 and JEV are disclosed in U.S. Pat. No. 5,494,671. U.S. Pat. No. 5,514,375 discloses various recombinant vaccinia viruses which express portions of the JEV open reading frame extending from **prM** to NS2B. These pox viruses induced

formation of extracellular particles that contain the processed M protein and the **E** protein. Two recombinant viruses encoding these JEV proteins produced high titers of neutralizing and hemagglutinin-inhibiting antibodies, and protective immunity, in. . . of these effects was greater after two immunization treatments than after only one. Recombinant vaccinia virus containing genes for the **prM**/M and **E** proteins of JEV conferred protective immunity when administered to mice (Konishi et al., Virology 180: 401-410 (1991)). HeLa cells infected with recombinant vaccinia virus bearing genes for **prM** and **E** from JEV were shown to produce subviral particles (Konishi et al., Virology 188: 714-720 (1992)). Dmitriev et al. reported immunization. . .

SUMM . . . 62: 3027-3031(1988)). Bray et al. (J. Virol. 63: 2853-2856 (1989)) also reported a recombinant vaccinia dengue vaccine based on the **E** protein gene that confers protective immunity to mice against dengue encephalitis when challenged. Falgout et al. (J. Virol 63: 1852-1860. . . 64: 4356-4363 (1990)) reported similar results. Zhang et al. (J. Virol 62: 3027-3031 (1988)) showed that recombinant baculovirus encoding dengue **E** and NS1 proteins likewise protected mice against dengue encephalitis when challenged. Other combinations in which structural and nonstructural genes were. . . (1989)). Also, monkeys failed to develop fully protective immunity to dengue virus challenge when immunized with recombinant baculovirus expressing the **E** protein (Lai et al. (1990) pp. 119-124 in F. Brown, R. M. Chancock, H. S. Ginsberg and R. Lerner (eds.). . .

SUMM . . . model (Phillpotts et al., Arch. Virol. 141: 743-749 (1996); Kochel et al., Vaccine 15: 547-552 (1997)). Plasmid DNA encoding the **prM** and **E** genes of SLEV provided partial protection against SLEV challenge with a single or double dose of DNA immunization. In these. . . et al., Arch. Virol. 141: 743-749 (1996)). In mice that received three intradermal injections of recombinant dengue-2 plasmid DNA containing **prM**, 100% developed anti-dengue-2 neutralizing antibodies and 92% of those receiving the corresponding **E** gene likewise developed neutralizing antibodies (Kochel et al., Vaccine 15: 547-552 (1997)). Challenge experiments using a two-dose schedule, however, failed. . .

SUMM [0023] There is therefore a need for vaccines or improved vaccines directed against flaviviruses such as **yellow fever virus**, dengue virus, JEV, SLEV and WNV which are inexpensive to prepare, present little risk to workers involved in their manufacture,. . .

SUMM . . . being incorporated within the cell, to synthesize the antigen. In an important aspect of the invention, the flavivirus can be **yellow fever virus** (YFV), dengue serotype 1 virus (DEN-1), dengue serotype 2 virus (DEN-2), dengue serotype 3 virus (DEN-3), dengue serotype 4 virus. . . (WNV), Powassan virus or any other flavivirus. In important embodiments of the present invention, the antigen can be the flavivirus **prM**/M protein, the **E** protein, or both. In particular, when the TU includes both the **prM**/M and **E** proteins, the host cell secretes subviral particles containing the **prM**/M and **E** antigens. In a further important aspect of the invention, the nucleic acid is a DNA molecule. In additional significant embodiments, the nucleic acid TU includes a control sequence disposed appropriately such that it operably controls the expression of the **prM**/M and **E** antigens and this control sequence can be the cytomegalovirus immediate early promoter. In an additional embodiment, the nucleotide sequence of. . .

SUMM . . . DEN-1, DEN-2, DEN-3, DEN-4, SLEV, JEV, WNV, Powassan virus or other flavivirus. In important embodiments, the antigen may be the **prM**/M protein, the **E** protein, or both the **prM**/M and the **E** proteins. In the latter case, the cell secretes subviral particles containing the **prM**/M and **E** antigens.

SUMM . . . be YFV, DEN-1, DEN-2, DEN-3, DEN-4, SLEV, JEV, WNV, Powassan virus or other flavivirus. Furthermore, the antigen may be the **prM**/M protein, the **E** protein, or both the **prM**/M and the **E** proteins. In the latter instance, the cell secretes subviral particles comprising the flavivirus **prM**/M and **E** antigens. These subviral particles are also referred to as noninfectious recombinant antigen (NRA). In important embodiments, the nucleic acid molecule. . . embodiments, the transcriptional unit additionally contains a control sequence disposed appropriately such that it operably controls the synthesis of the **prM**/M and **E** antigens when the nucleic acid is introduced into the cell of the subject. This control sequence can be the cytomegalovirus. . .

SUMM . . . JEV, WNV, Powassan virus or other flavivirus. In yet other important aspects of the method, the antigen may be the **prM**/M protein, the **E** protein, or both the **prM**/M and the **E** proteins. When the antigen is both the **prM**/M and the **E** proteins, the cell within the body of the subject, after incorporating the nucleic acid within it, secretes subviral particles comprising the flaviviral **prM**/M and **E** antigens. Additionally, in significant embodiments of the method, the vaccinating composition is administered to the subject in a single dose,. . . method, the transcriptional unit further includes a

control sequence disposed appropriately such that it operably controls the synthesis of the **prM**/M and **E** antigens and in a significant aspect of this embodiment, the control sequence is the cytomegalovirus immediate early promoter. Furthermore, the. . .

SUMM . . . acid, such as the cell of a subject to whom the vaccine is administered, produces subviral particles containing the flaviviral **prM**/M and **E** antigens. These particles mimic the immunogenic attributes of native flavivirus virions.

SUMM [0031] The present invention also provides noninfectious antigenic polypeptides, antigenic polypeptide fragments and NRA comprising the **prM**/M and/or **E** proteins of flaviviruses, wherein the transmembrane signal sequence is derived from a first flavivirus and the M and/or **E** proteins are derived from a second flavivirus. Further, the **prM**/M protein can comprise amino acid sequences from both the first and the second flaviviruses. "Chimeric" as used herein means any. . .

SUMM . . . the present invention can comprise the amino acid sequences defined herein, or that are known in the art, of the **prM**, M and/or **E** proteins of selected flaviviruses. The nucleic acids of this invention can comprise nucleotide sequence that encodes the **prM**, M and/or **E** proteins of selected flaviviruses.

SUMM [0035] The amino acid sequences of the present antigens can contain an immunoreactive portion of the **prM**, M and/or **E** antigen. These antigens may further be attached to sequences designed to provide for some additional property, such as to remove/add. . .

DRWD . . . (left and top) and non-structural (right and bottom) proteins. Cleavage by host cell signalase occurs simultaneously with translation at the **E** protein C-terminus, separating structural and non-structural regions. A subtilase-like cellular enzyme, furin, may be responsible for **prM** cleavage. Potential transmembrane domains of viral polyprotein are indicated by shaded areas.

DRWD . . . of oligonucleotides used in a reverse transcriptase-polymerase chain reaction (RT-PCR) (center) to construct the transcription unit for the expression of **prM-E** protein coding regions (bottom). Potential transmembrane domains of viral polyprotein are indicated by shaded areas.

DRWD . . . hormone polyadenylation signal and transcription termination sequence), ampicillin resistance gene and ColE1 origin of replication for selection and maintenance in **E**. coli. The f1 origin of replication for single-stranded rescue in **E**. coli cells, SV40 origin of replication (SV40 ORI), neomycin resistance coding region and SV40p(A) sequences were deleted from pCDNA3 to. . .

DRWD . . . cell culture was used as a positive control (JEV, left lane of each pair). JE HIAF (hyperimmune ascitic fluid); 4G2, anti-**E** monoclonal antibody; JM01, anti-M monoclonal antibody; NMAF (normal mouse ascitic fluid).

DRWD [0040] FIG. 5 shows a profile of the **E** antigen in a rate zonal sucrose gradient analysis prepared from the PEG precipitate of JE-4B cell culture medium with or. . .

DETD [0041] The invention encompasses nucleic acid transcriptional units which encode flaviviral antigenic proteins, such as the **prM**/M and **E** protein antigens. The nucleic acids function to express the **prM**/M and **E** protein antigens when the nucleic acid is taken up by an appropriate cell, especially when the cell is the cell. . .

DETD . . . of this invention can encode an immunogenic flavivirus antigen which can be from one or more of the following flaviviruses: **yellow fever virus**, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, Powassan. . .

DETD . . . nucleic acid of this invention can encode a signal sequence of Japanese encephalitis virus and an M protein and an **E** protein of West Nile virus, SLEV, YFV and/or Powassan virus. The nucleic acid can also encode an immunogenic antigen which can be an M protein of a flavivirus, an **E** protein of a flavivirus, both an M protein and an **E** protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an **E** protein of a flavivirus and/or both a portion of an M protein of a flavivirus and a portion of an **E** protein of a flavivirus. In a preferred embodiment, the isolated nucleic acid encodes both the M protein and the **E** protein of the flavivirus. Further, the nucleic acid of the invention can be DNA and can comprise nucleotide sequence SEQ. . .

DETD . . . a particular embodiment, the composition used to immunize a subject directs the synthesis of both the M protein and the **E** protein of a flavivirus and a cell within the body of the subject, after incorporating the nucleic acid within it, secretes subviral particles comprising the M protein and the **E** protein. Alternatively, the composition can comprise an M protein and/or **E** protein of a flavivirus or subviral particles comprising the M protein and **E** protein. In the methods of this invention, the immunizing composition can be administered to the subject in a single dose. . .

DETD . . . virus. The antigen can also be protein from dengue virus, St. Louis encephalitis virus, Japanese encephalitis virus, Powassan virus and/or **yellow fever virus**. In a further embodiment, the antigen comprises a **prM**/M protein comprising the transmembrane signal sequence from a first flavivirus and further amino acid sequence comprising the remainder of the **prM**/M protein from a second flavivirus, which can be from SLEV, JEV, YFV, WNV and/or Powassan virus.

DETD . . . West Nile virus antigen, dengue virus antigen, St. Louis encephalitis virus antigen, Japanese encephalitis virus antigen, Powassan virus antigen and/or **yellow fever virus** antigen.

DETD [0051] The antigen encoded by the nucleotide sequence of the TU can also be the **E** protein, which can be the **E** protein from West Nile virus, dengue virus, St. Louis encephalitis virus, Japanese encephalitis virus, Powassan virus and/or **yellow fever virus**.

DETD [0052] Additionally, the antigen encoded by the nucleotide sequence of the TU can be the M protein and the **E** protein, which can be from West Nile virus, dengue virus, St. Louis encephalitis virus, Japanese encephalitis virus, Powassan virus and/or **yellow fever virus**.

DETD [0053] As used herein, "M protein" or "pr/M protein" or "**prM**/M protein" means a flavivirus M protein or flavivirus **prM** protein. Examples include, but are not limited to, **prM** proteins comprising amino acid sequence from one or more flavivirus **prM** proteins, M proteins comprising no additional amino acid sequence and proteins comprising additional amino acid sequences which are processed in. . .

DETD . . . to be bound by theory, it is believed that an immunogenic response may arise from the generation of neutralizing antibodies (i.**e.**, a humoral immune response) or from cytotoxic cells of the immune system (i.**e.**, a cellular immune response) or both. As used herein, an "immunogenic antigen" is an antigen which induces an immunogenic response. . .

DETD . . . that encode specific gene products related to antigens of flaviviruses such as, but not limited to, WNV, JEV, dengue virus, **yellow fever virus** and SLEV. Although any nucleic acid may serve as a TU, in an important embodiment, the TU is DNA. Alternatively,. . .

DETD . . . the processing of the polyprotein. In particular, the presence of the pr sequence is important in preventing misfolding of the **E** protein. Thus, the presence of **prM** allows for assembly of JEV particles. Once the virion or particle is formed, the pr sequence can be cleaved from the **prM** protein to yield mature virus particles containing M proteins, although cleavage of the **prM** protein to yield M protein is not necessary to produce infectious particles. The **prM** sequences from many different, related flaviviruses are cleaved to but a low extent, but the flaviviruses themselves are nonetheless, infectious.. . .

DETD [0064] In one embodiment, the TU encoding flaviviral M and **E** proteins in the instant invention is DNA. In accord with the discussion in the preceding paragraph, this DNA comprises a nucleotide sequence which encodes the M protein, comprising the pre-M sequence, and a nucleotide sequence encoding the **E** protein. In this way, the intended gene products are enabled to form subviral particles within the cell. The pre-M sequence. . .

DETD . . . as a template for the synthesis of cDNA using reverse transcriptase. From the cDNA, a fragment containing the pre-M through **E** coding region (FIG. 2) is obtained by digestion with restriction nucleases known to cleave the cDNA appropriately to provide such. . .

DETD . . . into which a TU has been introduced. The TU of the present invention induces the intracellular biosynthesis of the encoded **prM**/M and **E** antigens. A suitable cell is one which has the capability for the biosynthesis of the gene products as a consequence. . . eukaryote cell. In particular embodiments of the present invention, the cell is a mammalian cell. In these cases, the synthesized **prM**/M and **E** protein gene products are available for use in analytical, or diagnostic applications, including preparation of antigen for use as a. . .

DETD [0070] In some circumstances, such as when the cell is a cultured mammalian cell, the **prM**/M and **E** antigens are secreted in the form of subviral particles. These are aggregates of **prM**/M and **E** proteins resembling live virus in surface ultrastructural morphology and immunogenic properties. Since the TU of the invention does not include. . .

DETD . . . to any promoter sequences, and terminator, if present. In any case, the TU induces the subject's cells to synthesize flaviviral **prM**/M and **E** gene products. Without wishing to be constrained by theoretical considerations, it is believed that the subject's cells produce subviral particles in vivo consisting of the **prM**/M and **E** antigens, just as has been found to occur with cultured mammalian cells in vitro. Such subviral particles, it is believed,. . . Again without wishing to be limited by theory, the resulting protective immunity may arise via either humoral or cellular immunity, i.**e.**, via either an MHC

class II- or class I-restricted mechanism, respectively, or by both mechanisms.

DETD . . . WNV or other flaviviruses by administering to them an effective amount of a TU comprising nucleic acid which encodes the **prM** and/or **E** antigens. The nucleic acid, after being incorporated into the cells of the subject, leads to the synthesis of the flaviviral **prM**/M and/or **E** antigens.

DETD . . . which comprises a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means a material that is not biologically or otherwise undesirable, i.**e**., the material may be administered to an subject along with the immunogenic material (i.**e**., recombinant flavivirus protein antigens or portions thereof) without causing any undesirable biological effects or interacting in a deleterious manner with. . .

DETD [0075] The TU may be administered to a subject orally, parenterally (**e**.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, intranasally, topically or the like. Delivery can also be directly to any area of the respiratory system (**e**.g., lungs) via intubation. The exact amount of the TU required will vary from subject to subject, depending on the species,. . .

DETD . . . parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, **e**.g., U.S. Pat. No. 3,610,795, which is incorporated by reference herein.

DETD . . . forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences (Martin, **E**. W. (ed.), latest edition, Mack Publishing Co., Easton, Pa.).

DETD . . . upon the subject. In order to undertake such a determination, the skilled artisan can assess the ability to induce flaviviral **prM**/M- and **E**-specific antibodies and/or flaviviral **prM**/M- and **E**-specific cytotoxic T lymphocytes present in the blood of a subject to whom the vaccine has been administered. One can also. . .

DETD . . . present invention can be used to elicit effective immune responses in a subject. Antigens for this purpose can comprise flavivirus **prM** protein, flavivirus M protein, flavivirus **E** protein or any combination thereof, including immunogenic fragments of the proteins. A particularly preferred embodiment is the use of the. . .

DETD . . . response. The term "suitable" is meant to include any substance which can be used in combination with the vaccine immunogen (i.**e**., flavivirus **prM** protein, flavivirus M protein, flavivirus **E** protein, or any combination thereof) to augment the immune response, without producing adverse reactions in the vaccinated subject. Effective amounts. . . suitable adjuvants can be chosen from the following group: mineral, vegetable or fish oil with water emulsions, incomplete Freund's adjuvant, **E**. coli J5, dextran sulfate, iron oxide, sodium alginate, Bacto-Adjuvant, certain synthetic polymers such as Carbopol (BF Goodrich Company, Cleveland, Ohio),. . . carrageenan, REGRESSIN (Vetrepharm, Athens, Ga.), AVRIDINE (N, N-dioctadecyl-N',N'-bis(2-hydroxyethyl)-propanediamine), long chain polydispersed β (1,4) linked mannan polymers interspersed with O-acetylated groups (**e**.g. ACEMANNAN), deproteinized highly purified cell wall extracts derived from non-pathogenic strain of Mycobacterium species (**e**.g. EQUIMUNE, Vetrepharm Research Inc., Athens Ga.), Mannite monooleate, paraffin oil and muramyl dipeptide.

DETD . . . Immunization can be carried out orally, parenterally, intranasally, intratracheally, intramuscularly, intramammarily, subcutaneously, intravenously and/or intradermally. The vaccine containing the flavivirus **prM** protein, flavivirus M protein and/or the flavivirus **E** protein can be administered by injection, by inhalation, by ingestion, or by infusion. A single dose can be given and/or repeated doses of the vaccine preparations, i.**e**. "boosters," can be administered at periodic time intervals to enhance the initial immune response or after a long period of. . .

DETD . . . the antibody. Such single chain antibodies are well known in the art and can be produced by standard methods. (see, **e**.g., Alvarez et al., Hum. Gene Ther. 8: 229-242 (1997)).

DETD . . . against the antigens of this invention which are synthesized from nucleic acid sequences encoding immunogenic amino acid sequences of the **prM**, M and/or **E** antigens of one or more flaviviruses and the signal sequence of a different flavivirus (**e**.g., JEV). Immunogenic peptides synthesized from the use of these chimeric constructs can easily be identified by use of methods well. . .

DETD [0094] The antibody or ligand of this invention can be bound to a substrate (**e**.g., beads, tubes, slides, plates, nitrocellulose sheets, etc.) or conjugated with a detectable moiety or both bound and conjugated. The detectable moieties contemplated for the present invention can include, but are not limited to, an immunofluorescent moiety (**e**.g., fluorescein, rhodamine), a radioactive moiety (**e**.g.,

^{32}P, ^{125}I, ^{35}S), an enzyme moiety (e.g., horseradish peroxidase, alkaline phosphatase), a colloidal gold moiety and a biotin moiety. Such conjugation techniques are standard in the art. . .

DETD . . . (3) contact the above with a secondary antibody bound to a detectable moiety which is reactive with the bound antibody (e.g., horseradish peroxidase enzyme or alkaline phosphatase enzyme); (4) contact the above with the substrate for the enzyme; (5) contact the. .

.

DETD . . . a competitive inhibition assay. Briefly, sample is contacted with an antigen of this invention which is bound to a substrate (e.g., an ELISA 96-well plate). Excess sample is thoroughly washed away. A labeled (e.g., enzyme-linked, fluorescent, radioactive, etc.) monoclonal antibody is then contacted with any previously formed antigen-antibody complexes and the amount of monoclonal. . .

DETD Preparation of Recombinant Plasmids Containing the Transcriptional Unit Encoding JEV **prM** and **E** Antigens.

DETD . . . silica membrane, was eluted in 80 μL of nuclease-free water, and used as a template for the amplification of JEV **prM** and **E** gene coding sequences. Primer sequences were obtained from the work of Nitayaphan et al. (Virology 177: 541-552 (1990)). A single. . .

DETD [0111] Plasmids containing the transcriptional unit encoding JEV **prM** and **E** proteins were prepared from these plasmids. The cDNA fragment containing the JEV **prM** and **E** coding regions in the recombinant plasmid pCDJE2-7 (nucleotide sequence, SEQ ID NO:10; amino acid sequence, SEQ ID NO:11), derived from. . .

DETD Evaluation of JEV **prM** and **E** Proteins Expressed by Various Recombinant Plasmids Using an Indirect Immunofluorescent Antibody Assay.

DETD [0113] To determine the influence of various promoter and poly(A) elements on the JEV **prM** and **E** protein expression, COS-1 and COS-7 cell lines were transiently transformed by an equal amount of pCDJE2-7 (SEQ ID NO: 10),. . .

DETD . . . a level of JEV antigens similar to that observed with pCDJE2-7 (Table 1). This result indicates that expression of JEV **prM** and **E** antigens by recombinant vectors is influenced only by the transcriptional regulatory elements. Neither the eukaryotic origin of replication nor the. . .

DETD [0116] Authenticity of the JEV **E** protein expressed by the JE-4B clone was demonstrated by epitope mapping by IFA using a panel of JEV **E**-specific murine monoclonal antibodies (Mab) (Kimura-Kuroda et al., J. Virol. 45: 124-132 (1983); Kimura-Kuroda et al., J. Gen. Virol. 67: 2663-2672. . .

DETD . . . (Nature 277: 680-685 (1970)). Proteins were transferred to a nitrocellulose membrane and immunochemically detected with polyclonal JEV HIAF, flavivirus cross-reactive anti-**E** Mab 4G2 (Henchal et al., Amer. J. Trop. Med. Hyg. 31: 830-836 (1982)), or mouse anti-**prM** peptide hyperimmune serum (JM01). FIG. 4 shows a comparison of the M and **E** proteins produced by JEV infected C6/36 and JE-4B COS-1 cells. Some nonspecific reactivity to **E** protein was observed in the normal mouse ascitic fluid and Jmol anti-peptide serum. Proteins identical in size to M and **E** were secreted in the subviral particles and could be detected by **E**-specific Mab 4G2 and **prM**-specific JM01 antiserum, respectively.

DETD . . . finding was reported by Konishi et al. (Virol. 188: 714-720 (1992)). These results show that rapidly sedimenting subviral particles containing **prM**/M and **E** could be disrupted by detergent treatment.

DETD [0128] Plasmid pCBJE1-14 provided the highest extent of seroconversion, i.e., antibody titer greater than 1:1600, achieving 80-100% at both ages of vaccination. Administration of pCDJE2-7 or pCIBJES 14 provided moderate. . . 3 days of age, and 100% when given at 3 weeks of age. Thus the nucleic acid TU's for JEV **prM** and **E** provided an extent of seroconversion better than a very high dose of the commercial vaccine, and unexpectedly high seroconversion in. . .

DETD Preparation of Recombinant Plasmids Containing the Transcriptional Unit Encoding WNV **prM** and **E** Antigens.

DETD . . . was eluted and suspended in 80 μl of nuclease-free water, and used as a template for the amplification of WNV **prM** and **E** gene coding sequences. Primer sequences were obtained from the work of Lanciotti et al. (Science 286: 2333-2337 (1999)). A cDNA. . .

DETD . . . prism 377 Sequencer (Applied Biosystems/Perkin Elmer, Foster City, Calif.) was used to confirm that the recombinant plasmid had a correct **prM** and **E** sequence as defined by Lanciotti et al. (Science 286: 2333-2337 (1999)).

DETD Immunochemical Characterization and Evaluation of WNV **prM** and **E** Proteins Expressed by pCBWN.

DETD [0137] Protein expression was detected using indirect immunofluorescence antibody assay (IFA), as described in Example 2. Flavivirus **E**-protein specific monoclonal antibody (Mab) 4G2, WNV mouse hyperimmune ascitic fluid (HIAF) and normal mouse serum (NMS) at 1:200 dilution in. . .

DETD [0140] WN virus-specific protein was detected by IFA on the transiently transformed COS-1 cells. **E**, **prM** and M proteins expressed in these

cells were secreted into the culture medium. WN virus antigen concentrated by PEG precipitation. . . WN virus-specific proteins produced by the transiently transformed COS-1 cells were detected by WN virus specific mouse HIAF or flavivirus **E** protein reactive Mab 4G2 in a Western blot analysis, using NMS as a negative serum control. The proteins displayed similar reactivity and identical molecular weights to the corresponding gradient purified virion **E**, **prM** and M protein derived from WN virus infected suckling mouse brain (SMB).

DETD . . . as lyophilized as β-propiolactone-inactivated sucrose-acetone extracts (Clarke et al., Am. J. Trop. Med. Hyg. 7: 561-573 (1958)). All recombinant proteins, **prM**, M and **E**, had a similar reactivity to that of the gradient-purified virion **E**, **prM** and M proteins.

DETD [0143] An Ag-capture ELISA employing flavivirus-group reactive, anti-**E** Mab, 4G2 and 6B6C-1, was used to detect NRA secreted into culture fluid of pCBWN transformed COS-1 cells. The antigen. . .

DETD . . . virus challenge (9-wk post immunization) had Nt antibody titers of 1:640 or 1:320. Pooled vaccinated mouse sera reacted only with **E** protein in the Western blot analysis.

DETD Preparation of Recombinant Plasmids Containing Coding Sequences for **Yellow Fever Virus** (YFV) or St. Louis Encephalitis Virus (SLEV) **prM** and **E** Proteins.

DETD . . . RNA Kit (Qiagen, Santa Clarita, Calif.). The viral RNA was used as a template for amplification of YFV or SLEV **prM** and **E** gene coding regions. Primers YFDV389 (nucleotide sequence, SEQ ID NO:4; amino acid sequence, SEQ ID NO:5), cYFDV2452 (SEQ ID NO:6),. . . from YFV strain TRI-788379 or SLEV strain 78V-6507. Recombinant plasmids pCDYF2 and pCDSLE4-3, which contained the nucleotide sequences of the **prM** and **E** coding regions for YFV or SLEV, respectively, were purified using an EndoFree.TM. Plasmid Maxi Kit (Qiagen), and used for in. . .

DETD . . . obtained which constitutively express YFV or SLEV antigenic proteins. Epitope mapping by IFA using a panel of YFV or SLEV **E**-specific Mabs indicated that the authentic **E** protein was expressed by the pCDYF2- or pCDSLE4-3-transformed COS-1 cells. A preliminary study indicated that 100% of three week-old female,. . .

DETD Preparation of Recombinant Plasmids Containing Coding Sequences for St. Louis Encephalitis Virus **prM** and **E** Antigens with JEV Signal Sequence.

DETD . . . suspended in 80 μl of nuclease-free water, and used as a template for the amplification of St. Louis encephalitis virus **prM** and **E** gene coding sequences. Primer sequences were obtained from the work of Trent et al. (Virology 156: 293-304 (1987)). A cDNA. . .

DETD . . . prism 377 Sequencer (Applied Biosystems/Perkin Elmer, Foster City, Calif.) was used to confirm that the recombinant plasmid had a correct **prM** and **E** sequence as defined by Trent et al. (Virology 156: 293-304 (1987)).

DETD Preparation of Recombinant Plasmids Containing Coding Sequences for **Yellow Fever Virus** (YFV) **prM** and **E** Proteins with JEV Signal Sequence.

DETD [0160] Genomic RNA was extracted from 150 μL of Vero cell culture medium infected with 17D-213 strain of **yellow fever virus** using the QIAamp.TM. Viral RNA Kit (Qiagen, Santa Clarita, Calif.). Extracted RNA was eluted and suspended in 80 μl of nuclease-free water, and used as a template for the amplification of **yellow fever virus** **prM** and **E** gene coding sequences. Primer sequences were obtained from the work of dos Santos et al. (Virus Research 35: 35-41 (1995)).. . .

DETD . . . prism 377 Sequencer (Applied Biosystems/Perkin Elmer, Foster City, Calif.) was used to confirm that the recombinant plasmid had a correct **prM** and **E** sequence as defined by dos Santos et al. (Virus Research 35: 35-41 (1995)).

DETD Preparation of Recombinant Plasmids Containing Coding Sequences for Powassan Virus **prM** and **E** Antigens with JEV Signal Sequence.

DETD . . . eluted and suspended in 80 μl of nuclease-free water, and used as a template for the amplification of Powassan virus **prM** and **E** gene coding sequences. Primer sequences were obtained from the work of Mandl et al. (Virology 194: 173-184 (1993)). A cDNA. . .

DETD . . . prism 377 Sequencer (Applied Biosystems/Perkin Elmer, Foster City, Calif.) was used to confirm that the recombinant plasmid had a correct **prM** and **E** sequence as defined by Mandl et al. (Virology 194:173-184, (1993)).

DETD [0167] A plasmid containing the dengue serotype 2 gene region from **prM** to **E** is to be constructed. The dengue serotype 2 **prM** and **E** genes (Deubel et al., Virology 155:365-377 (1986); Gruenberg et al., J. Gen. Virol. 69: 1301-1398 (1988); Hahn et al., Virology. . .

DETD [0168] The dengue serotype 2 nucleic TU vaccine encoding the gene region from **prM** to **E** prepared in Example 16 is to be suspended in a suitable pharmaceutical carrier, such as water for injection or buffered. . . second day. Passive protection by maternal antibody is to be assessed as indicated in Example 8.

TABLE 1

Transient expression of
JE **prM** and **E** proteins by various recombinant plasmids in two transferred cell lines

Vector	Recombinant	IFA intensity/percentage of antigen-positive cells*
Promotor	Intron	Poly (A). . .

CLM What is claimed is:

. . . nucleic acid of claim 1, wherein the immunogenic flavivirus antigen is of a flavivirus selected from the group consisting of **yellow fever virus**, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, Powassan. . .

. . . claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an **E** protein of West Nile virus.

. . . claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an **E** protein of **yellow fever virus**.

. . . claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an **E** protein of St. Louis encephalitis virus.

. . . claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an **E** protein of Powassan virus.

. . . of claim 1, wherein the antigen is selected from the group consisting of an M protein of a flavivirus, an **E** protein of a flavivirus, both an M protein and an **E** protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an **E** protein of a flavivirus and both a portion of an M protein of a flavivirus and a portion of an **E** protein of a flavivirus or any combination thereof.

9. The nucleic acid of claim 8, wherein the antigen is both the M protein and the **E** protein of a flavivirus.

. . . 19. The method of claim 18, wherein the flavivirus antigen is of a flavivirus selected from the group consisting of **yellow fever virus**, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, Powassan. . .

. . . of claim 18, wherein the antigen is selected from the group consisting of an M protein of a flavivirus, an **E** protein of a flavivirus, both an M protein and an **E** protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an **E** protein of a flavivirus and both a portion of an M protein of a flavivirus and a portion of an **E** protein of a flavivirus or any combination thereof.

21. The method of claim 20, wherein the antigen is both the M protein and the **E** protein of a flavivirus, and wherein a cell within the body of the subject, after incorporating the nucleic acid within it, secretes subviral particles comprising the M protein and the **E** protein.

. . . claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an **E** protein of West Nile virus.

. . . claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an **E** protein of **yellow fever virus**.

. . . claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an **E** protein of St. Louis encephalitis virus.

. . . claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an **E** protein of Powassan virus.

32. The nucleic acid of claim 1, wherein the antigen is a **yellow fever virus** antigen.

33. The method of claim 18, wherein the antigen is a **yellow fever**

virus antigen.

L6 ANSWER 5 OF 7 USPATFULL on STN
2000:174106 Subunit immonogenic composition against dengue infection.
Ivy, John, Kailua, HI, United States
Nakano, Eilen, Hon., HI, United States
Clements, David, Honolulu, HI, United States
Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
US 6165477 20001226
APPLICATION: US 1997-915152 19970820 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB . . . that cause significant morbidity in humans including the dengue (DEN) virus, Japanese encephalitis (JE) virus, tick-borne encephalitis virus (TBE), and **yellow fever virus** (YF). Flaviviruses are generally transmitted to vertebrates by chronically infected mosquito or tick vectors. The viral particle which is enveloped. . . by host cell membranes, comprises a single positive strand genomic RNA and the structural capsid (CA), membrane (M), and envelope (**E**) proteins. The **E** and M proteins are found on the surface of the virion where they are anchored in the membrane. Mature **E** is glycosylated and contains functional domains responsible for cell surface attachment and intraendosomal fusion activities. Problems have arisen in the art with respect to producing recombinant forms of the **E** glycoprotein that retain their native configuration and attendant properties associated therewith (i.**e**., ability to induce neutralizing antibody responses). To date, recombinantly produced **E** glycoproteins have suffered from a number of limitations including improper glycosylation, folding, and disulfide bond formation. The claimed invention has addressed these concerns by providing secreted recombinant forms of the **E** glycoprotein that are highly immunogenic and appear to retain their native configuration. Carboxy-terminally truncated forms of **E** containing the amino terminal 395 amino acids and a suitable secretion signal sequence were generated in Drosophila melanogaster Schneider cell. . .

SUMM . . . vectors. Flaviviruses are enveloped by host cell membrane and contain the three structural proteins capsid (C), membrane (M), and envelope (**E**). The **E** and M proteins are found on the surface of the virion where they are anchored in the membrane. Mature **E** is glycosylated, whereas M is not, although its precursor, **prM**, is a glycoprotein. Glycoprotein **E**, the largest structural protein, contains functional domains responsible for cell surface attachment and intraendosomal fusion activities. It is also a. . .

SUMM . . . aching muscles and joints, and rash. A fraction of cases, typically in children, results in more extreme forms of infection, i.**e**., dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Without diagnosis and prompt medical intervention, the sudden onset and rapid. . .

SUMM . . . products encoded by the single, long open reading frame are contained in a polyprotein organized in the order, C (capsid), **prM**/M (membrane), **E** (envelope), NS1 (nonstructural), NS2a, NS2b, NS3, NS4a, NS4b, and NS5 (Chambers, T. J. et al. Ann Rev Microbiol (1990) 44:649-688).. . . comparing the nucleotide sequence and the amino terminal sequences of the viral proteins. Subsequent to initial processing of the polyprotein, **prM** is converted to M during virus release (Wengler, G. et al. J Virol (1989) 63:2521-2526), and anchored C is processed. . .

SUMM . . . 162:167-180; DEN-3, Osatomi, K. et al. Virus Genes (1988) 2:99-108; Osatomi, K. et al. Virology (1990) 176:643-647; DEN-4, Zhao, B. **E**. et al. Virology (1986) 155:77-88; Mackow, **E**. et al. Virology (1987) 159:217-228). In addition, the compete genomic sequences of other flaviviruses are known (**e**.g., YF virus: Rice et al., Science (1985) 229:726-733).

SUMM . . . molecular structural features of the envelope protein of flaviviruses are found in Halstead, S. B. Science (1988) 239:476-481; Brandt, W. **E**. J Infect Disease (1990) 162:577-583; Chambers, T. J. et al. Annual Rev Microbiol (1990) 44:649-688; Mandl, C. W. et al. Virology (1989) 63:564-571; and Henchal, **E**. A. and J. R. Putnak, Clin Microbiol Rev (1990) 3:376-396.

SUMM Monoclonal antibodies (Mabs) directed against purified **E** of several flaviviruses DEN-2 (Henchal et al. Am J Trop Med Hyg (1985) 34:162-169, TBE (Heinz, F. X. et al.. . .

SUMM Although the primary amino acid sequence of the flavivirus **E** glycoprotein is variable (45-80% identity), all have twelve conserved cysteine residues, forming six disulfide bridges, and hydrophilicity profiles are nearly. . . studies, monoclonal antibody binding to purified proteolytic fragments, and analysis of neutralizing antibody escape mutants of Tick-Borne Encephalitis Virus, glycoprotein **E** was

divided into three antigenic domains (A, B, and C) and two transmembrane segments at its carboxy-terminus. See, for example,. . .

SUMM Recombinant dengue proteins have been expressed in several systems to date (see Putnak, R. A. (1994) Modern Vaccinology, **E**. Kurstak ed., Plenum Medical, New York, pp. 231-252, for review). Most efforts using Escherichia coli have yielded poor immunogen unable. . .

SUMM Several reports have described vaccinia-flavivirus recombinants expressing envelope protein as part of a polyprotein (**e**.g. C-**prM**-**E**-NS1; [Dengue] Zhao, B. G. et al. J Virol (1987) 61:4019-4022; Deubel, V. et al. J Gen Virol (1988) 69:1921-1929; Bray,. . . et al. J Virol (1991) 63:2853-2856; [YF] Hahn, Y. S. et al. Arch Virol (1990) 115:251-265), as a single protein (**e**.g. 100% **E**; [Dengue] Bray, M. et al., J Virol (1989) 63:2853-2856), or as polypeptides (**e**.g. 79% **E**-RKG; Men, R. et al. J Virol (1991) 65:1400-1407). The most successful recombinant vaccinia viruses, those capable of inducing neutralizing antibodies and protecting mice from virus challenge, were those which were secreted **E** extracellularly or accumulated **E** on the cell surface.

SUMM . . . appear to result from reduced production of the protein. It was also found that only truncations which contained 79% of **E** or less were secreted efficiently; **E** polypeptides equal to or larger than 81% **E** were not secreted efficiently.

SUMM Men et al. (1991, supra) constructed additional C-terminal truncations between 79% **E** and 81% **E** to map the amino acids responsible for the difference in secretion and immunoreactivity with HMAF of these two truncated **E** polypeptides. The results demonstrated that 79% **E** containing the additional tripeptide sequence RKG was also secreted. Although both 59% **E** and 79% **E**-RKG were secreted, only 79% **E**-RKG was detected at the cells' surface. The recombinant Vaccinia viruses containing various truncations were also used to immunize mice. Mice immunized with recombinants expressing 79% **E**-RKG or larger portions of the envelope protein were protected. However, except for 59% **E**, mice immunized with 79% **E** or a smaller product were only partially protected. The 59% **E** elicited high protection rates (>90%) comparable to 79% **E**-RKG and larger C-terminal truncated **E** polypeptides. Protection correlated with binding to HMAF.

SUMM . . . et al. Am J Trop Med Hyg (1991) 45:159-167; Deubel, V. et al. Virology (1991) 180:442-447). Baculovirus-expressed dengue and JE **E** glycoprotein elicited neutralizing antibodies, protected mice from a lethal dengue virus challenge, or both. In spite of these successes, the. . .

SUMM . . . antibodies in mice (Srivastava, A. K. et al. Acta Virol (1990) 34:228-238). Immunization of mice with a larger polypeptide (JE **E** amino acid 319 to NS1 amino acid 65) spanning the 8 kD peptide expressed in Escherichia coli as a fusion. . .

SUMM . . . Virol (1990) 71:2107-2114 identified two domains of the DEN-1 envelope protein: domain I which includes amino acids 76-93 of the **E** protein and domain II (equivalent to domain B) which includes amino acids 293-402. These domains were identified from deletion analysis using recombinant fusion proteins expressed in **E**. coli and reacted with antiviral monoclonal antibodies. Recombinant fusion proteins containing **E**. coli trpE sequences fused to the envelope protein (amino acids 1 to 412) elicited antibodies in mice which reacted with. . .

SUMM In addition, Mason, P. W. et al. (J Gen Virol (1989) 70:2037-2049) expressed a collection of **E**. coli trpE fusion proteins to segments of JE virus envelope protein spanning domain B. The trpE fusion proteins containing the smallest JE **E** fragments that retained immunoreactivity with a panel of neutralizing monoclonal antibodies included amino acid residues from methionine 303 through tryptophan. . .

SUMM Trirawatanapong, T. et al. Gene (1992) 116:139-150 prepared several truncated forms of dengue 2 envelope proteins in **E**. coli for epitope mapping, and mapped monoclonal antibody 3H5 to its corresponding epitope. This was first localized between amino acids. . . amino acids between positions 386 and 397. The mapping was apparently confirmed by the ability of a synthetic peptide containing **E** protein amino acids 386-397 to bind 3H5 specifically.

SUMM Megret, F. et al. Virology (1992) 187:480-491 prepared 16 overlapping fragments of DEN-2 envelope protein as trpE fusion products in **E**. coli for epitope mapping. The fusion proteins are produced intracellularly and obtained from the lysates. These products were used to. . .

SUMM . . . findings of Trirawatanapong et al. Gene (1992, supra), MAb 3H5 was unable to bind to trpE fusion proteins containing DEN-2 **E** amino acids 304-397, 298-385, or 366-424. The two exceptional MAbs in the findings of Megret et al. are MAbs 5A2. . .

SUMM . . . a form which mimics the native protein and is thus capable of eliciting an immune response. The only recombinantly produced **E** polypeptides containing the B domain that elicited a protective immune response in mice were expressed from Vaccinia and baculovirus vectors.. . .

SUMM . . . eliciting the production of neutralizing antibodies against dengue virus. In the illustrations below, the B domain of the envelope protein (**E**) is secreted from yeast by producing it in an expression vector containing the α-mating factor prepropeptide leader sequence (preproMFα_L). Peptide subunits representing 60% **E** and 80% **E** are secreted from Drosophila cells using the human tissue plasminogen activator secretion signal sequence for the propeptide (tPA$_L$) or from the homologous premembrane (**prM**) leader. The secreted products can easily be purified and prepared as a vaccine.

SUMM . . . virus. The vaccine contains, as active ingredient, the envelope protein of a dengue virus serotype or a subunit thereof. The **E** or subunit is secreted as a recombinantly produced protein from eucaryotic cells. The vaccine may further contain portions of additional dengue virus serotype **E** proteins similarly produced.

DRWD FIG. 13 shows the survival times of mice immunized with D. melanogaster Schneider cell-secreted 80% **E**.

DETD . . . envelope protein extending approximately from the Gly at position 296 to the Gly at position 395, and optionally including additional **E** sequence through position 413 of the protein, and other portions of **E**, i.e., 60% **E** and 80% **E** are effectively secreted by certain convenient eucaryotic recombinant hosts, in a form that permits processing to mimic the native conformation. . .

DETD . . . a peptide which spans from approximately Gly 296 to Gly 395 of the DEN-2 envelope protein, and optionally including additional **E** sequence through position 413 of the envelope protein. These positions are approximate; for example, Mandl (1989, supra) describes the generation of a tryptic fragment containing domain B which spans the amino acids of the TBE **E** protein from position 301 to 396. The sequences described in the present application represent the envelope protein from dengue Type. . . extensions do not interfere with the immunogenic effectiveness or secretion of the B domain. In one embodiment, such extensions are minimal--i.e., not more than six additional amino acids--at either the N-terminus or the C-terminus, or distributed between these termini; preferably no. . .

DETD . . . domain includes at least portions of the region extending to amino acid 413, the additional region may confer additional functions, e.g., enhancing immunogenicity by providing a helper T cell epitope. The form of domain B which includes positions about 296-413 is. . .

DETD Other portions of the **E** protein illustrated below are self-explanatory. 80% **E** is the N-terminal 80% of the protein from residue 1 to residue 395. 60% **E** represents the corresponding shorter sequence. These subunits are produced from vectors containing the DNA encoding the mature protein, or along with the **prM** fusion which results in secretion of the 80% or 60% **E** per se.

DETD . . . ovary cells. Other insect cells may also be used in conjunction with baculovirus based vectors. The B domain or 60% **E** or 80% **E** must be produced as a correctly processed protein and secreted.

DETD . . . invention. An additional preferred embodiment employs Drosophila cells and the human tissue plasminogen activator leader sequence for secretion of 60% **E** or 80% **E** as well as domain B. Envelope protein subunits that represent N-terminal portions of truncated protein may also be secreted from the homologous **prM** fusion. Other secretion signal peptides or secretion leader pre/pro peptides, such as those associated with invertase or acid phosphatase of. . .

DETD The properly processed **E** protein or subunit is recovered from the cell culture medium, purified, and formulated into vaccines. Purification and vaccine formulation employ. . .

DETD . . . can themselves be used as passive vaccines. For production of passive vaccine, a suitable mammalian subject is immunized with the **E** protein or subunit of the invention and antibodies are either recovered directly as a composition from the antisera or indirectly. . .

DETD In addition to use in vaccines or in the generation of passive vaccines, the mature recombinant **E** protein and subunits of the invention may be used as analytical reagents in assessing the presence or absence of antidengue. . .

DETD Thus, the secreted protein, such as 60% **E**, 80% **E** or B domain may be adsorbed onto solid support and the support then treated with a sample to be tested. . .

DETD In addition, both the mature peptides, such as domain B and 60% **E** or 80% **E** of the invention and the antibodies immunoreactive with it can be used in standard purification procedures as affinity reagents. Thus,. . .

DETD In the examples below, particular subunits of the dengue Type 2 envelope protein, in particular 60% **E**, 80% **E** and domain B are illustrated as representative of effective subunits of the envelope protein. For the 60% **E** and 80% **E** constructs in general, secretion can be obtained from constructions designed to express the prME subunit fusion. The mature N-terminus of. . . subunits were fused to a heterologous leader, such as the human tissue plasminogen activator leader sequence,

or to the homologous **prM** sequence, the mature form of the truncated envelope protein is secreted. The secreted truncated Es are expressed at high levels. . . secreted into the medium. The products are glycosylated and processed to an endo-H resistant form. The secreted form of truncated **E** produced cotranslationally with **prM** generally represents about 20-30% of the total protein in the medium. Furthermore, based upon reactivity with conformationally sensitive monoclonal antibodies, using a ELISA and immunofluorescence formats, the secreted **E** products are shown to have a native conformation. Immunization of mice with crude medium from transformed cells expressing **prM** truncated **E** induces a potent virus-neutralizing response.

DETD . . . wild-type sequence. There are no nucleotide differences in the Capsid and preMembrane protein-encoding portions and there are four in the **E** encoding portion.

DETD In the **E** gene, three of the four mutations are silent; S1 has G instead of A at position 1314, T rather than. . . conservative, mutagenesis studies of other viral structural proteins (Coller, B. G. et al. (1994) Mutagenesis Studies on the Predicted VP2 **E**-F Loop of Coxsackievirus B3, Abstract, 13th Annual Meeting of the American Society for Virology) have demonstrated that even relatively conservative. . .

DETD Various **E** gene subclones were obtained which represented the amino-terminal 90% of the envelope, 80% of the envelope, 60% of the envelope. . . et al. at its carboxy end which can be included in some forms of the domain B of the invention, **e**.g., DomB+T.

DETD The portion of the genome that encodes 80% of the envelope protein (80% **E**) was amplified using the Polymerase Chain Reaction, primers D2E937p and D2E2121m, and plasmid pC8 (Hahn et al. (1988, supra) as. . .

DETD In this notation of the primers, the virus serotype is first indicated (D2 for DEN-2), then the corresponding dengue gene--i.**e**., in this case envelope, **E**, is noted. Then is noted the number in the dengue cloned sequences of FIGS. 2 or 3 for the first dengue nucleotide in the 5'-3' direction of the oligonucleotide, i.**e**., using the numbering of Hahn et al. (1988, supra), and finally the notation shows whether the oligonucleotide primes the plus. . .

DETD The D2E2121m primer placed two stop codons after the 395th codon of **E**. The 80% **E** amplified cDNA fragment was digested at the XbaI sites in the cloning adapters and cloned into the NheI site of pBR322 to obtain p29D280E. Double-strand sequence for 80% **E** was determined, which identified a single silent PCR-introduced mutation at nucleotide 2001 (AAC/Asn to AAT/Asn).

DETD A subclone representing domain B was obtained from the 80% **E** subclone by oligonucleotide-directed mutagenesis. In the mutagenesis, stop codons and restriction endonuclease sites were inserted between domain C- and domain. . . FIG. 4, to avoid a high AT content in the mutagenic oligonucleotide, the stop codons defining the carboxy-terminus of 60% **E** containing domains A and C were positioned four codons upstream of the beginning of domain B, i.**e**., following Lys291. The original and altered nucleotide sequences of the mutagenized region and the corresponding amino acid translation are shown. . .

DETD To perform the mutagenesis, a 580 bp BamHI fragment spanning domain B from the pBR322-80% **E** clone p29D280E was subcloned into pGEM3Zf (Promega) to yield p29GEB2. (See FIG. 5.) This BamHI fragment encodes the 3' end. . .

DETD The cloned cDNA fragments encoding B domain and 80% **E** were inserted into expression vectors so as to maintain the translational frame of fusions to secretion leaders as described below.. . .

DETD . . . cloning sites, and use the TRP1 gene as a selectable marker. They contain sequences derived from pBR322 to provide an **E**. coli origin of replication, the ampicillin resistance gene, and sequences derived from the 2-micron plasmid of S. cerevisiae to enable. . .

DETD . . . a fusion with the $MF\alpha_L$. This construct includes those sequences that lie between domain B and the transmembrane anchor of **E**. This region contains a potential T cell epitope (Mandl et al. J Virol (1989) 63:564-571) and additional hydrophobic sequences, a. . .

DETD The domain B+stem cDNA fragment was constructed in **E**. coli cloning vectors by combining the domain B cDNA fragment and the 3' end of a 90% **E** clone. As introduced in Example 1, an **E** gene subclone representing the amino terminal 90% of **E** was constructed from DEN-2 PR159/S1 cDNA plasmid pC8 of Hahn et al. (1988, supra) using the PCR. The 90% **E** polypeptide contains all of **E** except for the C-terminal membrane anchor comprising two transmembrane domains. The 90% **E** cDNA clone was made as follows. The 90% **E** fragment was amplified by the PCR using pC8 as template and primers D2E937p and D2E2271m. The sequence of D2E937p is. . .

DETD . . . of #, and the two primers positioned useful restriction enzyme sites at both ends of the fragment. The PCR-amplified 90% **E** cDNA fragment was made blunt at both ends and cloned into the SmaI site of a modified pUC13 cloning vector,. . .

DETD Combining domain B and the 90% **E** 3' end made use of a unique AflIII

restriction enzyme site found in most pUC-like cloning vectors and a unique AflIII site in domain B sequences. This combining was accomplished by first subcloning the 90% **E** fragment from pVZ90E into pBluescript to reverse the orientation of 90% **E** relative to the vector sequences, yielding pBS90E. Then, p29GEB24PS, containing domain B sequences in PGEM (Example 1), and pBS90E were. . . AflIII, and the vector-domain-B5' fragment and the domain-B3'-stem-vector fragment from the two digestions, respectively, were purified, ligated, and recovered in **E**. coli yielding pBS-Bstem.

DETD . . . flaviviruses, when assayed using a similar ELISA format. Flavivirus infected murine sera tested include, Japanese Encephalitis virus, Tick-Borne Encephalitis virus, **Yellow Fever virus**, Saint Louis Encephalitis virus, West Nile virus, three viral isolates of dengue serotype 1, two viral isolates of dengue serotype. . .

DETD To perform the sandwich enzyme immunoassay, 100 µl of anti-Dengue monoclonal antibody 9D12 or 3H5 (Henchal, **E**. A. et al., Am J Trop Med Hyg (1985) 34:162-169) was used to coat microtiter wells. The monoclonal antibodies were. . .

DETD **E**. DomB Immunizations for Hybridoma Generation: Six BALB/c mice were immunized with 87 µg of unconjugated DomB or 174 µg KLH-conjugated. . .

DETD Production of 60% **E** and 80% **E** in Drosophila

DETD . . . Drosophila metalothionein gene, the human tissue plasminogen activator signal and the SV-40 early polyadenylation signal, the nucleotide sequences encoding 80% **E, prM** 80% **E**, 60% **E** and **prM** 60% **E** are inserted and the resulting vectors used to transform Schneider cells as described in Example 9. The mature truncated forms. . .

DETD Expression of 80% **E** in Saccharomyces cerevisiae

DETD An expression vector (pLS6-80% **E**) was constructed for secretion of the N-terminal 80% (codons 1-395) of the DEN-2 PR-159 S1 envelope glycoprotein (80% **E**) from S. cerevisiae. The 80% **E** DNA sequences were obtained from plasmid p29D280E, described in example 1, by restriction endonuclease digestion with both BglII and SalI.. . . the BglII and SalI sites of pLS6, a yeast expression vector described in example 2. The resulting recombinant plasmid, pLS6-80% **E**, contains truncated **E** as a translationally in-frame fusion to the leader region of mating-factor α (MFα), a secreted yeast protein. The MFα leader. . . Kex2p, a golgi protease, and subsequent trimming of N-terminal (Glu/Asp)Ala dipeptides by dipeptidyl aminopeptidase (DPAP). The herein described $MF\alpha_L$ -80% **E** fusion was made such that processing of the $MF\alpha_L$ propeptide and trimming of a GluAla dipeptide results in 80% **E** with eight additional N-terminal amino acids derived from sequences present in the multiple cloning site of the pLS6 vector or in the PCR primer-adaptor used to synthesize the 80% **E** cDNA (see below).

DETD . . . ACC ATG...GGA TAA

TAG

- Met-18aa Ala-65aa Glu Ala Phe Arg Ser Arg Val Pro Gly Thr Met_1..

.Gly_{395} End End

- 80%**E**

- .tangle-solidup. .tangle-solidup. .tangle-solidup

- Signalase Kex2p DPAP

DETD After confirming the DNA sequence of the ligated junctions of expression vector pLS6-80% **E**, the recombinant DNA was transformed into S. cerevisiae strain GL43 (MATa trplΔ1 ura3-52 pep4::URA3; SmithKline Beecham) according to standard protocols. . .

DETD In order to test for expression and secretion of 80% **E**, several transformants were grown as small-scale cultures (5 ml medium in 17×150 mm tubes). Single colonies were used to inoculate. . . a Dengue gene insert secreted no proteins recognized by the anti-domain B serum, while the major immunoreactive band from pLS6-80% **E** medium had a relative mobility matching that of other recombinant 80% **E** proteins (see Example 17). The pLS6-80% **E** medium also contained a minor immunoreactive species with an apparent molecular weight 6-8 kD higher than 80% **E**; this is likely to be unprocessed MFα propeptide-80% **E**. The pLS6-80% **E** cellular protein extract contained many immunoreactive polypeptides not observed in negative control cells, two of which match the secreted products discussed above. A Coomassie-stained protein corresponding to recombinant 80% **E** could not be identified in either the pLS6-80% **E** secreted sample or the total cellular protein sample. This indicates the relatively low levels of recombinant protein expression in these. . .

DETD Construction of Expression Vector DPIC9-80% **E** and Secretion of 80% **E** by P. pastoris Expressing $MF\alpha_L$ -80% **E**

DETD The expression vector constructed to secrete 80% **E** from P. pastoris was engineered to express amino acids 1-395 of the DEN-2 PR-159 S1

envelope glycoprotein as a fusion to the MFα_L. The DNA sequences encoding 80% **E** were obtained from the clone p29D280E, described in example 1, by digestion with the restriction enzymes SmaI and SalI. The. . . isolated fragment was treated with the Klenow DNA polymerase I fragment enzyme to make the SalI end blunt. This 80% **E** fragment was then cloned into the Pichia expression vector pPIC9 (Invitrogen, San Diego, Calif.) which contains the MFα secretion leader. . . (MFα_L) sequence, SnaBI, EcoRI, and NotI cloning sites, and uses the HIS4 gene as a selectable marker. The described 80% **E** fragment was ligated with pPIC9 plasmid DNA that was previously digested with the restriction enzyme SnaBI. The orientation and genetic integrity of the resulting gene fusion expression vector, pPIC9-80% **E**, was confirmed by restriction digestion and DNA sequence analysis. The organization, partial nucleotide and predicted amino acid sequences of the MFα_L -80% **E** fusion gene are shown below:

DETD . . . GCC TTTAGATCTCGAGTACCCGGGACC ATG...GGA TAA
(SEQ ID NO:21 and SEQ ID NO:22)
Met-18aa-65aa
Glu Ala
PheArgSerArgValPr
oGlyThr Met_1
...Gly_{395}
END
80%**E**
- .tangle-solidup. .tangle-solidup. .tangle-solidup.
- Signalase
Kex2p DPAP

DETD . . . are indicated. The dengue sequences are indicated in bold type. The Met_1 residue is the N-terminal amino acid of the **E** glycoprotein and Gly_{395} is residue 395 from the amino terminal end of the envelope glycoprotein. The expression of a recombinant. . .

DETD The pPIC9-80% **E** expression vector was transformed into spheroplasts of P. pastoris strain GS115 (his4) and transformants were selected for their ability to. . . for transformation were obtained from Invitrogen (San Diego, Calif.). Transformants were tested for their ability to express and secrete 80% **E** by growing selected clones in small cultures (5 to 50 ml). The transformants were first grown to saturation (24 to. . . medium, a unique staining band of approximately 50 kD is present in the EndoH treated lanes of all the pPIC9-80% **E** transformants. Immunoprobing with anti-domain B serum (see example 6) detected a smear ranging from 50 to 90 kD in the. . . of the corresponding immunoreactive band were detected in EndoH treated samples of cellular protein samples. The approximately 50 kD 80% **E** product produced by the MFα_L -80% **E** construct is consistent with the approximate molecular weight as determined by SDS-PAGE of other recombinant 80% **E** proteins (see Example 17). The amount of secreted 80% **E** in the culture medium is about 1% of the total secreted protein as estimated by the intensity of the Coomassie staining band detected. In one liter cultures, the amount of 80% **E** secreted into the culture medium was determined to be 500 ng/ml by use of a sandwich ELISA method.

DETD Construction of Expression Vector pMttbns-80% **E** and Secretion of 80% **E** by Drosophila melanogaster Schneider Cells Expressing tPA_L -80% **E**

DETD The expression vector constructed to secrete 80% **E** from Drosophila melanogaster tissue culture cells included the sequences encoding the DEN-2 PR159/S1 envelope glycoprotein amino acids 1-395. The DNA sequences for 80% **E** were obtained from the clone p29D280E (described in Example 1) by digestion with the restriction enzymes BglII and SalI. The released 80% **E** fragment was cloned into the BglII plus XhoI-digested D. melanogaster expression vector pMttΔXho. The expression vector pMttΔXho is a derivative. . . make pMttΔXho, in which the BglII and another XhoI restriction endonuclease sites are unique. This construction resulted in the 80% **E** fragment being fused to the tPA_L sequence. During normal maturation of tissue plasminogen activator the 20 amino acid prepeptide region. . . amino acid propeptide region is enzymatically removed in the Golgi. The genetic integrity of the gene fusion expression vector, pMtt80% **E**, was confirmed by restriction digestion and DNA sequence analysis. The nucleotide and predicted amino acid sequences of the tPA_L -80 **E** fusion gene are shown below:

DETD . . . 20 aa - 11 aa -Gly Ala Arg Ser Arg Val Pro Gly Thr Met_1 ... Gly_{395} END
- pre.tangle-solidup. pro-tPA.tangle-solidup.
80%**E**

DETD The selection plasmid, pCOHygro (SmithKline Beecham), carries the **E**. coli hygromycin B phosphotransferase gene under the transcriptional control of a D. melanogaster copia transposable element long terminal repeat and. . . multiple copies of the co-transfected gene of

interest. Drosophila melanogaster Schneider cells (ATCC, Rockville, Md.) were cotransfected with the pMtt80% **E** and pCOHygro plasmids at a ratio of 20:1 using the calcium phosphate coprecipitation method (Gibco BRL). Transformants were selected by. . . Coomassie staining and immunoprobing of Western blots. The Coomassie blue-stained SDS-PAGE gels shows that the approximately 50 kD secreted 80% **E** product is one of the predominant proteins in the unconcentrated medium, comprising as much as 20% of the total protein.. . . revealed a single immunoreactive polypeptide of approximately 50 kD present in unconcentrated medium. In addition, immunoblots revealed that the 80% **E** produced by the tPA_L -80% **E** construct was slightly larger than that obtained upon expression of a tPA_L -prM80% **E** construct (described in detail in Example 17). This additional mass may owe to the nine adaptor amino acids at the amino terminus of 80% **E** (GARSRVPGT-(SEQ ID NO:25)80% **E**) when expressed from pMtt80% **E** versus 80% **E** expressed from pMttprM80% **E** (Example 17). The tPA propeptide, if not proteolytically removed, may also contribute to the additional molecular weight of 80% **E** expressed from pMtt80% **E**.

DETD Subcloning of Dengue prM100% **E** and prM80% **E** cDNAs and Mutagenesis of **E** Secretion Signal-encoding Sequence (mutSS)

DETD A cDNA clone of DEN-2 PR159/S1 designed to encode the preMembrane, Membrane, and Envelope genes (prM100% **E**) was constructed by PCR amplification essentially as described in Example 1 for the subcloning of 80% **E**. This cDNA clone includes nucleotides 439 to 2421 of the DEN-2 genome. The dengue cDNA fragment was generated using synthetic. .

.

DETD The PCR-generated prM100% **E** cDNA fragment was digested with the restriction endonuclease XbaI and ligated into the XbaI site of pBluescript SK+ (Stratagene, San. . .

DETD To generate a cDNA subclone representing prM80% **E**, a 794 bp BamHI-SalI fragment from p29prME13 representing the envelope carboxy terminal-encoding fragment was removed. This fragment was replaced with. . . confirmed by restriction digestion and DNA sequence analysis to encode amino acids 1 through 395 of the envelope glycoprotein following **prM**.

DETD Expression of the prM80% **E** cDNA in S. cerevisiae (Example 15) demonstrated absence of proteolytic processing between the **prM** and 80% **E** proteins in this yeast. To improve processing of **E** from **prM**, oligonucleotide-directed mutagenesis was performed to alter the naturally occurring signalase cleavage site between the **prM** and **E** proteins. Based on the algorithm of Von Heijne (1986, Nucl. Acids Res. 14:4683-4690), the natural DEN-2 **E** secretion signal peptide receives a poor predictive score for its function as a secretion signal. The algorithm of von Heijne. . . signal peptides range from 3.5 to 17, with a mean of .about.10. The score for the secretion signal peptide of **E** of DEN-2 PR159/S1 is 5.2, near the lower end of the range for signal peptides. In the mutagenesis, the sequence. . .

DETD To perform the mutagenesis, a 1,122 bp SmaI-HindIII fragment spanning the **prM-E** signalase cleavage site from the p29prME13 cDNA clone was subcloned into pAlterl (Promega, Madison, Wis.) to yield the plasmid pAltSmaH3prME. The 1,122 bp SmaI-HindIII fragment contains all of **prM** and 611 bp of the **E** sequence. The HindIII site is a naturally occurring site within the **E** sequence that is located at nucleotide 1547 of the genomic sequence. The mutagenized clone, pAltSmaH3prME(mutSS), was verified by DNA sequence. . .

DETD Construction of Expression Vectors pLS6-prM80% **E** and pLS6-**prM**(mutSS)80% **E**, Expression of $MF\alpha_L$ -prM80% **E** and $MF\alpha_L$ -**prM**(mutSS)80% **E** in Saccharomyces cerevisiae, and Secretion of 80% **E** by Saccharomyces cerevisiae Expressing $MF\alpha_L$ -**prM**(mutSS) 80% **E**

DETD . . . Example 2) that had been digested with BglII and SalI. The structure of the resulting gene fusion expression vector, pLS6-prM80% **E**, was confirmed by restriction digestion and DNA sequence analysis. The nucleotide and predicted amino acid sequences of the $MF\alpha_L$ -prM80% **E** fusion gene are shown below:

DETD . . . TTTAGATCTCGAGTACCCGGGACCATG TTT ...ACA ATG
...GGA TAA

- Met-18aa-65aa Glu Ala PheArgSerArgValProGlyThrMet Phe_1...Thr.sub
.166 Met_1...Gly_3
95 End

- .tangle-solidup. .tangle-solidup. .tangle-solidup.
prM 80%**E**
- Signalase Kex2p
DPAP

DETD . . . dengue sequences are indicated in bold. The Phe_1 and Thr_{166} residues are the N-terminal and C-terminal amino acid residues of **prM**, respectively. The Met_1 residue is the N-terminal amino acid of the envelope glycoprotein and Gly_{395} is residue 395

from the. . .

DETD The pLS6prM80% **E** plasmid was transformed into Saccharomyces cerevisiae strain GL43 (MATa ura3-52 trp1Δ1 pep4:URA3) and screened for 80% **E** expression as described in Example 1. Proteins secreted into the culture medium as well as total cellular proteins were treated. . . protein revealed a HMAF immunoreactive band of approximately 90 kD suggesting that the recombinant product had not been processed to **prM** and **E**. Probing of companion Western blots with polyclonal antisera that recognized the MFα leader peptide (from J. Rothblatt, Dartmouth University) confirmed. . . the product recognized by the anti-DEN2 HMAF was identical to that recognized by anti-MFα serum, demonstrating that the $MF\alpha_L$ -prM80% **E** fusion protein was not processed into its individual components ($MF\alpha_L$, **prM**, and 80% **E**).

DETD The unsuccessful processing of **E** from **prM** in the $MF\alpha_L$ -prM80% **E** fusion protein may be an obstacle to the proper folding and secretion of **E**. To assess whether the optimized dengue signal sequence (see Example 16) facilitated the processing of the envelope protein at the **prM-E** junction, the altered **E** signal sequence from pLS6prM(mutSS)100E-TGA was introduced into pLS6prM80% **E** to create plasmid pLS6prM(mutSS)prM80% **E**. This procedure replaced the native **E** signal sequence (Pro-Ser-Met-Thr_1 -Met+1 (SEQ ID NO:34)) with the optimized **E** signal sequence (Gly-Ala-Gln-Ala_{-1} -Gln+1 (SEQ ID NO:34)).

DETD . . . fragment between plasmids pAlterSmaH3prME(mutSS) (see Example 14) and pLS6prM100E. DNA sequencing of pLS6prM(mutSS)100E-TGA identified an unintended TGA stop codon within **E** downstream of the mutated secretion signal. To transfer the altered secretion signal encoding sequence to pLS6prM80% **E** and to separate the cDNA fragment containing the altered secretion signal of **E** from the TGA stop codon, a BglII-EcoNI fragment from pLS6prM(mutSS)100E-TGA, encompassing **prM** and the first 430 nucleotides of **E** and lacking the TGA stop codon, was transferred to plasmid pLS6prM80% **E** which had been similarly digested to yield the expression plasmid pLS6prM(mutSS)80% **E**. The sequence of the expression plasmid was confirmed by restriction digestion and DNA sequence analysis. The nucleotide and predicted amino acid sequences of the $MF\alpha_L$ -**prM** junction are identical to the sequences listed above.

DETD . . . unsupplemented minimal medium (see Example 11). Transformants were cultured, induced, and evaluated as described above for the non-mutated $MF\alpha_L$ -prM80% **E** transformants. Proteins secreted into the culture medium as well as total cellular proteins were treated with $EndoH_f$ prior to separation. . . a novel Coomassie staining band. Immunoprobing with anti-DEN2 HMAF and anti-DomB antiserum, however, revealed a small amount of processed immunoreactive **E** protein in the medium. The size of the immunoreactive protein (approximately 50 kD) was similar to the secreted protein from pLS6-80% **E** expression vector. Evaluation of intracellular expression of the fusion protein containing the optimized secretion signal by SDS-PAGE and Western blot. . . the transformed cells produce immunoreactive product recognized by anti-DEN2 HMAF and anti-DomB antiserum. Unlike the immunoreactive product seen in pLS6prM80% **E** transformants, the immunoreactive band found in pLS6(mutSS)prM80% **E** transformants was not recognized by $MF\alpha_L$ anti-serum suggesting that processing had occurred at the **prM-E** junction. Thus, the mutagenesis of the signalase cleavage site resulted in greatly enhanced processing of the $MF\alpha_L$ -prM80% **E** product at the **prM-E** junction.

DETD Construction of Expression Vector pPIC9-**prM**(mutss)80% **E** and Secretion of 80% **E** by P. pastoris Expressing $MF\alpha_L$ -**prM**(mutss) 80% **E**

DETD The expression vector constructed to express preMembrane-mutated secretion signal- 80% Envelope (pPICprM(mutSS)80% **E**) in P. pastoris from a single continuous open reading frame utilized the DEN-2 PR159/S1 **prM** and **E** gene sequences described above (Example 14). The plasmid, pPIC9-**prM**(mutSS)80% **E**, was constructed by transferring a **prM**(mutSS)80% **E** fragment from the S. cerevisiae expression plasmid pLS6prM(mutSS)80% **E** into pPIC9. The P. pastoris expression vector pPIC9 (Example 4) and the S. cerevisiae expression vector pLS6 (Example 2) both. . .

DETD Prior to transferring the **prM**(mutSS)80% **E** cDNA fragment, sequences encoding extraneous amino acids and an extraneous XhoI site at the $MF\alpha_L$ -**prM** fusion were first removed. This was accomplished by digesting pLS6-**prM**(mutSS)80% **E** with restriction endonuclease XhoI and XmaI into which was ligated a synthetic oligonucleotide duplex (5'-TCGAGAAGAGAGAAG-3' (SEQ ID NO:36) and 5'-CCGGCTTCTCTCTTC-3'. . . required for the cDNA fragment transfer from pLS6 to pPIC9. The

nucleotide and predicted amino acid sequence at the MFα_L -**prM** fusion junction from pLS6-**prM**(mutSS)80% **E** and pLS6Δ-**prM**(mutSS)80% **E** are:

DETD MFα_L -**prM** junction of pLS6-**prM**(mutSS)80%**E**

```
-         XhoI                              XhoI    XmaI
- ATG ...CTC GAG AAA AGG GAG GCC TTTAGATCTCGAGTACCCGGGACCATG TTT..
                                                              (SEQ ID
                                                              NO:38 and
                                                              SEQ ID
. . . Met
                                                              ...Leu Glu
                                                              Lys Arg
                                                              Glu Ala
                                                              PheArgSerAr
                                                              gValProGlyT
                                                              hrMet
                                                              Phe1..
-                        .tangle-solidup.       .tangle-solidup.
                                                                -
                                                                  Kex2p
                                                               DPAP
                                                                -
                                                              MFα.s
                                                              ub.L -prM
                                                              junction
                                                              of
                                                              pLS6.increm
                                                              ent.-prM(mu
                                                              tSS)80%E
                                                                -
                                                               XhoI
- ATG ...CTC GAG AAA AGG GAG GCC GGGACCATG TTT.. (SEQ ID NO:40 and SEQ
                                                              ID NO:41)
                                                                -. . .
```

DETD To construct the clone pPIC9-**prM**(mutSS)80% **E**, a XhoI-SalI fragment encoding **prM**(mutSS)80% **E** sequences was obtained from pLS6Δ-**prM**(mutSS)80% **E** and was inserted into the pPIC9 vector that had been digested with Xhol. The genetic integrity of the expression plasmid, pPIC9-**prM**(mutSS)80% **E**, was confirmed by restriction digestion.

DETD The pPIC9-**prM**(mutSS)80% **E** expression vector was transformed into spheroplasts of P. pastoris strain GS115 (his4), and His+ transformants were selected for their ability to grow on minimal medium without histidine supplementation. The transformants were screened for expression and secretion of 80% **E** as described in Example 12. No unique Coomassie staining bands were detected in the culture medium of either non-EndoH$_f$ or EndoH$_f$ treated samples (similar to that observed for culture medium from pPIC9-80% **E** transformants--see Example 12). Western immunoblots of proteins from the culture medium probed with anti-domain B serum detected multiple bands with. . . corresponding immunoreactive protein were detected in EndoH treated samples of cellular proteins. We estimate that the amount of secreted 80% **E** in the culture medium is less than 1% of the total amount of secreted protein based on the observation that. . .

DETD Construction of pMttbns-prM80% **E** and Secretion of 80% **E** by Drosophila melanogaster Schneider cells Expressing tPA$_L$ -prM80% **E**

DETD . . . in Drosophila melanogaster Schneider 2 tissue culture cells, DNA sequences encoding these proteins were obtained by digestion of the p48BSprM80% **E** clone (described in Example 14) with the restriction enzymes BglII and SalI. This fragment was cloned into the unique BglII. . .

DETD . . . ID NO:42 and SEQ ID NO:43)

```
ATG ..... GGAGCCAGATCTCGAGTACCCGGGACCATG TTT ..ACA     ATG  ..GGA
   TAA
 Met-20 aa- -11 aa-GlyAlaArgSerArgValProGlyThrMet Phe1..Thr166
Met1..Gly395  END
```

pre.tangle-solidup.pro-tPA.tangle-solidup.

prM 80%**E**

DETD . . . sequences are indicated in bold type. The Phe$_1$ and Thr$_{166}$ residues are the N-terminal and C-terminal amino acid residues of **prM**, respectively. The Met$_1$ residue is the N-terminal amino acid of envelope glycoprotein and Gly$_{395}$ is residue 395 from the amino. . .

DETD As described previously in Example 13, Schneider 2 cells were cotransfected with pMttprM80% **E** DNA at ratios of 1:1, 5:1, and 20:1 relative to pCOHygro DNA. Transformants were induced with 200 μM $CuSO_4$ and expression of prM80% **E** was examined at various times

after induction. Proteins secreted into the culture medium as well as cellular proteins were separated. . . Western blot analysis. This .about.50 kD immunoreactive band is roughly the same size as the secreted EndoH-treated product from pLS6-80% **E** transformed yeast cells (Example 11) and slightly smaller than the secreted 80% **E** from pMttbns80% **E**-transformed D. melanogaster Schneider cells (Example 13), suggesting the Envelope protein had been processed away from the preMembrane protein. (The size discrepancy between 80% **E** secreted by pMtt80% **E** and pMttprM80% **E** Schneider cells is discussed in Example 13.) Polyclonal antisera to the pr portion of **prM** (from Peter Wright, Monash University, Australia) did not recognize the .about.50 kD protein, confirming that the 80% **E** produced in the transfected cells was processed from **prM**. In fact, no evidence of a higher molecular weight band that might correspond to unprocessed prM80% **E** was detected in any sample, suggesting that the proteolytic processing of **prM** from **E** is extremely efficient in Schneider cells. The fate of the **prM** portion of the fusion remains unresolved as no distinct immunoreactive band was detected by probing with the anti-pr antisera.

DETD The secreted 80% **E** glycoprotein was partially purified (judged by the presence of a single major band on a sliver stained SDS-PAGE gel) and. . . NaCl. The 150 mM NaCl eluant was separated on an SDS-PAGE gel and electro-transferred to Immobilon-P membrane (Millipore). The 80% **E** band was excised, and the N-terminal amino acids were determined by Edman sequencing. Two amino acid sequences were obtained. One,. . . expected sequence, Met-Arg-Cys-Ile-Gly-Ile (SEQ ID NO:46), supporting the interpretation that the .about.50 kD secreted immunoreactive glycoprotein is correctly processed 80% **E** of DEN-2.

DETD Sensitivity of the secreted 80% **E** to endoglycosidases was evaluated by molecular weight shift of the protein in SDS-PAGE and Western immunoblots following endoglycosidase treatment. Resistance of the secreted 80% **E** to Endoglycosidase H_f (Endo H_f ; New England Biolabs) and sensitivity to N-glycosidase F (PNGase F; New England Biolabs) digestion. . .

DETD . . . the unconcentrated medium, comprising as much as 20% of the total secreted protein. Estimates of the concentration of the 80% **E** product in unconcentrated medium based upon sandwich ELISA assays (described in detail in Example 7) and Coomassie blue staining range. . . polyclonal anti-dengue 2 hyperimmune mouse ascites fluid (DEN-2 HMAF; from R. Putnak, WRAIR) demonstrated that the amount of secreted 80% **E** produced by the transfectants increased over time from day 1 post induction to 7 days post induction. The amount of 80% **E** detected intracellularly in the transfectants correlated with the cotransfection ratio, but the increase in intracellular 80% **E** with time was not as dramatic as for secreted 80% **E**, suggesting efficient secretion of 80% **E** and accumulation in the medium.

DETD Induction of Anti-Dengue 2 Antibodies in Mice by Pichia pastoris-secreted 80% **E**

DETD P. pastoris cells transformed with pPIC-80% **E** (described in Example 12) were induced with 0.5% methanol and the medium was collected after 40 hours of induction (for. . . Webster outbred mice (Simonsen) were immunized by intraperitoneal (I.P.) injection with 100 μg total protein of the crude concentrated 80% **E** medium with or without complete Freund's adjuvant. Controls for this experiment included a negative control medium prepared from a non-recombinant P. pastoris culture as described above for the 80% **E** medium. Protein precipitation was observed during the concentration of the negative control medium, consequently the final protein concentration of the concentrated medium was lower than that from the 80% **E** medium. (For this reason, 12.5 μg of total protein in Freund's complete adjuvant was used for immunization with the negative. . .

DETD . . . given viral inoculum. Results from the ELISA and PRNT assays are summarized in Table 4. The P. pastoris expressed 80% **E** induces a potent anti-DEN2 response in mice, with ELISA titers of up to 1:102,400. The titers obtained in the presence. . .

DETD TABLE 4

Induction of Anti-DEN2 Immune Response in Mice Immunized with P. pastoris-expressed 80% **E**

mouse	antigen	adjuvant	3° titer ELISA	4° titer ELISA	4° titer $PRNT_{80}$
30-1	Saline	Freund's	<1:50	<1:100	
30-2			<1:50	<1:100	
30-3.	.	.	<1:100	<1:100	
34-5	medium		<1:100	1:100	
36-1	100 μg	Freund's	>1:6400	1:25,600	<1:10

36-2 Pichia 1:6400 1:25,600 <1:10
36-3 80% **E** >1:6400 1:25,600 <1:10
36-4 total 1:100 1:100 <1:10
36-5 medium 1:6400 1:102,400 <1:10
37-1 100 μg None <1:100 1:100
37-2 Pichia 1:1600 1:6400
37-3 80% **E** 1:100 1:400
37-4 total 1:400 1:6400
37-5 medium 1:100 <1:100

DETD Induction of Dengue Virus-neutralizing Antibodies by Immunizing Mice with 80% **E** Secreted by Drosophila melanogaster Schneider Cells Expressing tPA_L -prM80% **E** and tPA_L -80% **E**

DETD Schneider cells, transformed with pMtt-prM80% **E** and pMtt-80% **E** expressing the tissue plasminogen activator leader fusion proteins tPA_L -prM80% **E** and tPA_L -80% **E**, respectively (Described in detail in Examples 17 and 13, respectively), were cultured in serum-free medium (Excell; JRH Biosciences) and induced. . . centrifuge and the media were filtered through a 0.2 μm cellulose acetate filter (Nalgene). The media containing the recombinant 80% **E** were concentrated 20-fold using centrifugal concentrators (Centriprep 30; Amicon) and assayed by ELISA (described in detail in Example 7) and. .

DETD . . . summarized in Tables 5 and 6. In both series of immunizations, the mice immunized with the crude media containing 80% **E**, expressed cotranslationally with **prM** or independently without **prM**, developed high titer, virus-neutralizing antibodies. These titers are higher than any previously reported titers for any immunogen produced from any. .

DETD TABLE 5

Immune Response of Mice Immunized with Crude Drosophila Media Containing Dengue 2 Virus 80% **E** Expressed as a **prM** 80% **E** Fusion

mouse	antigen	adjuvant	ELISA	2° titer[a] ELISA	3° titer[b] ELISA	titer[c] $PRNT_{80}$ titer[c]
20-1		Saline				
		Freund's				
			<1:50			
				<1:50	<1:100	
.	.	.	<1:50	<1:100	<1:10	
21-3	medium		<1:50	<1:50	<1:100	<1:10
21-4			<1:50	<1:50	<1:100	<1:10
21-5			<1:50	<1:50	<1:100	<1:10
22-1	**prM** 80% **E**	Freund's	1:3200	>1;25,600	1:102,400	1:2560
22-2	secreted		>1:800	>1:6,400	1:25,600	1:2560
22-3	medium		>1:200	>1:1,600	1:25,600	1:2560
22-4			<1:50	>1:102,400.	.	.

DETD TABLE 6

Immune Response of Mice Immunized with Crude Drosophila Media Containing Dengue 2 Virus 80% **E** Expressed with or without **prM**

mouse	antigen	adjuvant	ELISA	2° titer[a] ELISA	3° titer[b] ELISA	Final titer[c] ELISA
25-1	Saline	Freund's	<1:50	>1:50	<1:10	
25-2			<1:50	>1:50.	.	.
27-5			>1:6400	1:409,600	<1:500	
28-1	100 μg	Freund's	DEAD	NT	NT	
28-2	Drosphila		>1:6400	1:102,400	1:8000	
28-3	**prM** 80% **E**		>1:6400	1:409,600	1:8000	
28-4	total		>1:6400	1:102,400	1:4000	
28-5	medium		>1:6400	1:102,400	1:1000	
29-1	100 μg	Freund's	1:6400	1:6400	1:500	
29-2	Drosophila		1:6400	1:102,400	1:4000	
29-3	80% **E**		<1:100	1:25,600	1:1000	
29-4	total		>1:6400	1:25,600	1:8000	
29-5	medium		>1:6400	1:102,400	1:4000	

[a] Determined following the 2nd injection.
[b]. . .

DETD Protection from Dengue Virus Challenge by Immunizing Mice with 80% **E** Secreted by Drosophila melanogaster Schneider Cells Expressing tPA_L

-prM80% **E** or tPA_L -80% **E**

DETD Schneider cells, transformed with pMtt-prM80% **E** and pMtt-80% **E** expressing the tissue plasminogen activator leader fusion proteins tPA_L -prM80% **E** and tPA_L -80% **E** respectively (described in detail in Examples 17 and 13, respectively), were cultured in serum-free medium (Excell; JRH Biosciences) and induced. . . of each preparation (corresponding to 70 μg total protein from negative control medium, 230 μg total protein from tPA_L -80% **E** medium, and 150 μg total protein from tPA_L -prM80% **E** medium) were used to subcutaneously inoculate groups of 10 mice each, using Alum as adjuvant. An identical second dose was. . .

DETD . . . the remaining two mice recovered by day 15 post-challenge. In contrast, of the 10 mice immunized with the tPA_L -80% **E**, only four exhibited any symptoms of infection, and eight of 10 survived the challenge. Similarly, nine of 10 mice immunized with tPA_L -prM80% **E** survived the challenge, although seven of these mice exhibited mild symptoms of infection during the monitoring period. These survival data are illustrated in FIG. 13, and show that both Drosophila-expressed 80% **E** antigens efficiently protected mice from viral challenge. These results emphasize the utility of the Drosophila cell expressed 80% **E** dengue immunogens as vaccine candidates.

DETD Construction of DEN-2 N-terminal 60% **E** and prM60% **E** cDNA Fragments

DETD A subclone encoding the N-terminal 60% of **E** was constructed using p29GEB24PS and p29D280E. Example 1 of the parent application--describes construction of p29GEB24PS. Plasmid p29GEB24PS holds a BamHI fragment insert containing, in part, DEN-2 **E** sequences (nucleotides 1696-2121) starting at a DEN-2 genomic BamHI site and ending with the Gly395 codon, followed immediately by two. . .

DETD The N-terminal 80% **E** insert in p29D280E was then converted to a 60% **E** insert by replacing a restriction fragment encoding the 3' end of 80% **E** with a restriction fragment from p29GEB24PS encoding the 3' end of 60% **E**. To accomplish this, DNA of p29GEB24PS was digested with BamHI, the .about.590 bp BamHI fragment was isolated by agarose gel. . . p29D280E prepared as follows. Plasmid p29D280E was digested with BamHI, which cuts the BamHI site (dengue nucleotides 1696-1701) within 80% **E**, and with SalI, which cuts immediately 3' of 80% **E** and also within the vector, pBR322, 422 base pairs distal to the 3' end of the 80% **E** fragment. Following ligation, the desired product, a plasmid containing the cDNA encoding the N-terminal 60% of **E** in pBR322 (p29D260E), was recovered by transformation of **E**. coli with the ligation mixture and screening transformant colonies for plasmids of the appropriate size and restriction digestion pattern. Proper. . .

DETD To construct a cDNA encoding **prM** and the amino terminal 60% of **E** (prM60% **E**), we used a strategy identical to that used to construct prM80% **E** (Example 14). The prM100% **E** plasmid, p29prME13, was digested with BamHI and SalI to release the 794 bp 3' end fragment of **E**, which was then replaced with the 119 bp BamHI-SalI fragment encoding a 40% carboxy-end truncation of **E** from p29D260E. The resulting truncated cDNA clone, p48BSprM60E, encodes a **prM**-60% **E** fusion ending with Lys291 of **E** and was confirmed by restriction digestion and DNA sequence analysis.

DETD Construction of Expression Vector pLS6-60% **E** and Secretion of 60% **E** by Saccharomyces cerevisiae Expressing MFα-60% **E**

DETD An expression vector (pLS6-60% **E**) was constructed for secretion of the N-terminal 60% (codons 1-291, 60% **E**) of the DEN-2 PR-159 S1 envelope glycoprotein from S. cerevisiae. The 60% **E** DNA sequences were obtained from plasmid p29D260E, described in example 21, by restriction endonuclease digestion with both BglII and SalI.. . . subcloned into the BglII and SalI sites of pLS6, a yeast expression vector described in example 2. The $MF\alpha_L$ -60% **E** fusion was made such that processing of the MFα propeptide and trimming of a Glu-Ala dipeptide results in 60% **E** with eight additional N-terminal amino acids encoded by sequences present in the multiple cloning site of the pLS6 vector and the **E** gene PCR primer adaptor (see below).

DETD . . . - Met-18aa Ala-65aa Glu Ala Phe Arg Ser Arg Val Pro Gly Thr Met_1...

Lys_{291} End End

\- .tangle-solidup. .tangle-solidup. .tangle-solidup.

60%**E**

\- Signalase Kex2p DPAP

DETD After confirming the DNA sequence of the ligated junctions of expression vector pLS6-60% **E**, the recombinant DNA was transformed into S. cerevisiae strain GL43 (MATa trp1Δ1 ura3-52 pep4::URA3; SmithKline Beecham) according to standard protocols. . .

DETD In order to test for expression and secretion of 60% **E**, several transformants were grown in small-scale cultures (5 ml medium in 17×150 mm tubes). Single colonies were used to inoculate. . .

DETD . . . control yeast carrying the expression vector without a Dengue

gene insert secreted no proteins recognized by anti-DEN2 HMAF, while pLS6-60% **E** medium contained several immunoreactive species. The major band presumably represents full-length 60% **E** since its apparent molecular weight is approximately 10 kD less than that of recombinant 80% **E**. A protein band comigrating with this immunoreactive material was visible in Coomassie-stained gels of proteins secreted by pLS6-60% **E** transformants, but this band was absent in medium of negative control transformants. The immunoblot of proteins secreted by pLS6-60% **E** transformants evidenced a minor band of apparent molecular weight 6-8 kD larger than 60% **E**; this likely represents unprocessed MFα propeptide-60% **E**. The cellular protein extract of pLS6-60% **E** transformants contained many immunoreactive polypeptides not observed in negative control cells; two of these match the secreted products discussed above.

DETD Construction of Expression Vectors pLS6-prM60% **E** and pLS6-**prM**(mutSS)60% **E**, Expression of $MF\alpha_L$ -prM60% **E** and $MF\alpha_L$ -**prM**(mutSS)60% **E** in Saccharomyces cerevisiae, and Secretion of 60% by Saccharomyces cerevisiae Expressing $MF\alpha_L$ -**prM**(mutSS)60% **E**

DETD . . . Example 2) that had been digested with BglII and SalI. The structure of the resulting gene fusion expression vector, pLS6-prM60% **E**, was confirmed by restriction digestion and DNA sequence analysis. The N-terminal $MF\alpha_L$ -**prM** fusion amino acid sequence of the $MF\alpha_L$ -prM60% **E** fusion protein are identical to those described for $MF\alpha_L$ -**prM**(mutSS)80% **E** fusions (Example 15), while the C-terminal amino acid of the fusion protein is Lys_{291} of the dengue envelope glycoprotein.

DETD The pLS6-prM60% **E** plasmid was transformed into S. cerevisiae strain GL43 (MATa ura3-52 trp1Δ1 pep4::URA3) and screened for 60% **E** expression as described in Example 11. Proteins secreted into the culture medium as well as cellular proteins were treated with. . . analyzed by both Coomassie staining of polyacrylamide gels and immunoprobing of Western blots. Similar to the expression of secreted 80% **E** from pLS6-prM80% **E** (Example 15), secreted 60% **E** was not detected on Coomassie stained polyacrylamide gels nor Western blots. An immunoblot of intracellular protein confirmed that the construct ($MF\alpha_L$ -prM60% **E**) was expressed, but the fusion product had not been processed to **prM** and **E** (evaluation was performed as described in Example 15).

DETD Because the dengue **E** signal sequence itself may limit processing of the prME fusion proteins, expression of $MF\alpha_L$ -**prM**(mutSS)60% **E** was evaluated. The BglII-EcoNI fragment from pLS6-**prM**(mutSS)100% **E**-TGA encoding the altered secretion signal peptidase cleavage site (see Example 15) was used to replace the homologous fragment from the pLS6-prM60% **E** to produce pLS6-**prM**(mutSS)60% **E**. The sequence of the expression plasmid was confirmed by restriction digestion and DNA sequence analysis. Plasmid pLS6prM(mutSS)60% **E** was transformed into the S. cerevisiae GL43 strain and transformants were selected as described in Example 11.

DETD . . . were cultured, induced, and evaluated as described in Examples 11 and 15. In contrast to the expression of $MF\alpha_L$ -prM60% **E**, Western blot analysis of total intracellular proteins from pLS6-**prM**(mutSS)60% **E** transformants demonstrated that the transformed cells produce an approximately 40 kilodalton product recognized by anti-DEN2 HMAF. For analysis of secreted proteins, media from induced cultures were concentrated, treated with endoglycosidase H_f, and analyzed on Western blots for **E** antigen. A small amount of processed 60% **E** could be identified in the culture medium upon immunoprobing with anti-DEN2 HMAF. Thus, the mutagenesis of the signalase cleavage site resulted in greatly enhanced processing of the $MF\alpha_L$ -**prM**(mutSS)60% **E** product at the **prM-E** junction which produced secretion of a processed 60% **E** from the $MF\alpha_L$ -**prM**(mutSS)60% **E** in S. cerevisiae.

DETD Construction of Expression Vector pPIC9-60% **E** and Secretion of 60% **E** by P. pastoris Expressing $MF\alpha_L$ -60% **E**

DETD The expression vector constructed to secrete 60% **E** from P. pastoris, pPIC9-60% **E**, included the DEN-2 PR-159 S1 envelope glycoprotein amino acids 1-291. As a precursor to this 60% **E** expression vector, a modified pLS6-60% **E** plasmid (pLS6-alt60% **E**), encoding a fusion between $MF\alpha_L$ and the amino terminal 60% of the dengue envelope, was constructed that encodes fewer non-dengue amino acids between the $MF\alpha_L$ and **E** segments. The 60% **E** cDNA fragment from pLS6-alt60% **E** was then transferred to pPIC9.

DETD The sequences in pLS6-alt60% **E** encoding the dengue **E** protein amino terminus were derived from pLS6-2×80E, which encodes a tandemly arrayed dimer of 80% **E** that are linked by a synthetic linker peptide.

To convert pLS6-2×80E to pLS6-alt60% **E**, pLS6-2×80E was digested with EcoNI, which cuts within the first member of the 80% **E** dimer, and SalI, which cuts immediately downstream of the dimer, thereby removing the 3' portion of the first member of. . . 80E dimer and the second member of the 80E dimer entirely. In its place was ligated an EcoNI-SalI fragment from pLS6-**prM**(mutSS)60E (see Example 23), encoding the 3' portion of 60% **E**, to complete construction of pLS6-alt60% **E**.

DETD The minimizing of non-dengue codons between $MF\alpha_L$ and **E**, found in pLS6-2×80E, were preserved in pLS6-alt60% **E**, and, subsequently, in pPIC9-60% **E**. The codons between $MF\alpha_L$ and **E** were minimized, owing to use of the XmaI site in p29D280E (XmaI is the first adaptor restriction enzyme site upstream of **E**; see Example 1) and the StuI site in pLS6. To ligate the XmaI 5' end of **E** to the StuI site in the $MF\alpha_L$, the XmaI site was treated with Klenow polymerase to make the end blunt. . .

DETD To transfer 60% **E** from pLS6-alt60% **E** to pPIC9, pLS6-alt60E was digested by XhoI plus SalI, which released a fragment that includes a portion of the MFα leader and the entire 60% **E** coding region. This fragment was ligated with the Pichia expression vector pPIC9 (Invitrogen, San Diego, Calif.; described in Example 12). . . complete MFα leader sequence including the Kex2 cleavage site. The genetic integrity of the resulting gene fusion expression vector, pPIC9-60% **E**, was confirmed by restriction digestion and DNA sequence analysis. The partial nucleotide and predicted amino acid sequences of the $MF\alpha_L$ -60% **E** fusion gene are shown below:

DETD . . . are indicated. The dengue sequences are indicated in bold type. The Met_1 residue is the N-terminal amino acid of the **E** glycoprotein and Lys_{291} is residue 291 from the amino terminal end of the envelope glycoprotein. The expression of a recombinant. . .

DETD The pPIC9-60% **E** expression vector was transformed into spheroplasts of P. pastoris strain GS115 (his4) and transformants were selected for their ability to. . . for transformation were obtained from Invitrogen (San Diego, Calif.). Transformants were tested for their ability to express and secrete 60% **E** by growing selected clones in small cultures (5 ml). The transformants were grown to saturation (24 to 36 hrs.) in. . . Mass.) and separated by SDS-PAGE. Western immunoblots probed with DEN-2 HMAF indicated that the recombinants expressed significant levels of 60% **E**. Protein gels analyzed by Coomassie staining also showed strong levels of 60% **E** expression and secretion.

DETD Construction of Expression Vector pPIC9-**prM**(mutSS)60% **E** and Secretion of 60% **E** by P. pastoris Expressing $MF\alpha_L$ -**prM**(mutSS)60% **E**

DETD To construct clone pPIC9-**prM**(mutSS)60% **E**, a strategy identical to that describe in Example 16 was used. Clone pLS6-**prM**(mutSS)60% **E** (described in Example 23) was digested with restriction endonuclease XhoI and XmaI and sequences within the $MF\alpha_L$ and dengue cloning. . . an extraneous XhoI site, to preserve a critical XhoI site, and to regenerate the Kex2 protease process site. The XhoI-SalI **prM**(mutSS)60% **E** fragment from pLS6Δ-**prM**(mutSS)60% **E** was ligated into the unique XhoI site of pPIC9. The nucleotide and amino acid sequences at the N-terminus of the fusion protein are identical to that shown in Example 16 for pLS6Δ-**prM**(mutSS)80% **E**. The structure of the Pichia expression vector pPIC9-**prM**(mutSS)60% **E** was confirmed by restriction digestion and DNA sequence analysis.

DETD The pPIC9-**prM**(mutSS)60% **E** expression vector was transformed into spheroplasts of P. pastoris strain GS115 (his4), and transformants were selected and evaluated as described. . .

DETD Construction of pMttbns-prM100% **E**, Expression of tPa-prM100% **E** by Drosophila melanogaster Schneider Cells, and Induction of a Virus Neutralizing Response by Immunizing Mice with tPa-prM100% **E** Membrane Preparations

DETD . . . and SEQ ID NO:52)

ATG ..... GGAGCCAGATCTCGAGTACCCGGGACCATG TTT ..ACA ATG ..GCC TAA

Met-20 aa-
-11 aa-GlyAlaArgSerArg
ValProGlyThrMet
Phe_1..Thr_{166}
Met_1..Ala_{495}
END

pre.tangle-solidup.pro-tPA.tangle-solidup.

prM

100%**E**

DETD . . . sequences are indicated in bold type. The Phe_1 and Thr_{166} residues are the N-terminal and C-terminal amino acid residues of **prM**, respectively. The Met_1 residue is the N-terminal

amino acid of envelope glycoprotein and Ala_{495} is residue 495 from the amino. . .

DETD As described previously in Example 13, Schneider 2 cells were cotransfected with pMttprM100% **E** DNA at ratios of 1:1, and 5:1, relative to pCOHygro DNA and selected for growth in medium containing 300 μg/ml hygromycin. Transfectants were induced with 200 μM $CUSO_4$ and expression of prM100% **E** was examined at various times after induction. Proteins secreted into the culture medium as well as cellular proteins were separated. . . This .about.60 kD immunoreactive band comigrated with viral Envelope derived from dengue-2 infected mosquito C6/36 cells suggesting the recombinant 100% **E** protein had been processed away from the premembrane protein. Polyclonal antisera to the pr portion of **prM** (from Peter Wright, Monash University, Australia) recognized a .about.20 kD protein, which comigrated with viral **prM**, confirming that the 100% **E** produced in the transfected cells was processed from **prM**. In fact, no evidence of a higher molecular weight band that might correspond to unprocessed prM100% **E** was detected in any sample, suggesting that the proteolytic processing of **prM** from **E** is extremely efficient in Schneider cells. No 100% **E** was detected in the culture medium indicating that 100% **E** remains anchored in cell-associated membranes.

DETD Immunoblots probed with anti-DEN-2 HMAF demonstrated that the amount of intracellular 100% **E** produced by the transfectants increased over time from day 1 post induction to 7 days post induction. The amount of 100% **E** detected intracellularly in the transfectants correlated with the cotransfection ratio. Sensitivity of the intracellular 100% **E** to endoglycosidases was evaluated by molecular weight shift of the protein in SDS-PAGE and Western immunoblots following endoglycosidase treatment. Partial resistance of the recombinant 100% **E** to Endoglycosidase H_f (Endo H_f ; New England Biolabs) digestion indicated that the product contains N-linked glycosylation, and that the. . .

DETD Schneider cells, transformed with pMtt-prM100% **E** expressing the tissue plasminogen activator leader fusion protein tPA_L -prM100% **E** were cultured in serum-free medium (Excell; JRH Biosciences) and induced by addition of $CuSO_4$ to a final concentration in the. . . Negative control cells transformed with pCOHygro only (see Example 13) were cultured, induced, and harvested as described for the prM100% **E**-expressing cells. The immunogens were assayed by Western blot prior to immunization of mice.

DETD Outbred Swiss Webster mice (Simonsen) were immunized intraperitoneally (I.P.) with 75 μg total protein of the prM100% **E** membrane preparation in Freund's complete adjuvant. Control animals were immunized with either 75 μg of total protein from the negative. . .

DETD . . . for the ELISA and PRNT assays are summarized in Table 7. The mice immunized with the membrane preparation containing 100% **E** developed virus-neutralizing antibodies despite the crude nature of the immunogen, suggesting the utility of these immunogens as efficacious vaccine candidates.

DETD TABLE 7

Immune Response of Mice Immunized with Recombinant Dengue prM100% **E** Membrane Preparation

mouse	antigen	adjuvant	2° titer[a] ELISA	3° titer[b] ELISA	final titer $PRNT_{80}$ ELISA titer[b]
20-1	Saline	Freund's	<1:50	<1:50. . .	<1:50 <1:50 <1:100 <1:10
23-3			<1:50	<1:50	<1:100 <1:10
23-4			<1:50	<1:50	<1:100 <1:10
23-5			<1:50	<1:50	<1:100 <1:10
24-1	**prM** 100% **E**	Freund's	1:200	1:1,600	>1:25600 1:320
24-2	membrane		<1:50	>1:1600	>1:1600 1:320
24-3			<1:50	>1:6000	>1:1600 1:80
24-4			<1:50	>1:6000	>1:25600. . .

DETD . . . - #C CCA CCA ACA GCA GGG
192
Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Il - #e Pro Pro Thr Ala Gly
50 - # 55 - # 60
- - ATA TTA AAA AGA TGG GGA. . . - #T AAG ACA AAG GAC GGC
432
Ser Arg Gln Glu Lys Gly Lys Ser Leu Leu Ph - #e Lys Thr Lys Asp Gly
130 - # 135 - # 140
- - ACG AAC ATG TGT ACC CTC. . .

DETD . . . - #A ATG GAT CTG GAA AAA
1872
Asp Gly Ser Pro Cys Lys Ile Pro Phe Glu Il - #e Met Asp Leu Glu Lys
610 - # 615 - # 620
- - AGA CAT GTT TTG GGC CGC. . . - #C GTC ACA GAT AAC GTG
2400
Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Ph - #e Val Thr Asp Asn Val
785 7 - #90 7 - #95 8 -
#00
- - CAT ACA TGG ACA. . . - #A GAG AAA GCT TCT TTC
2976
Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Il - #e Glu Lys Ala Ser Phe
980 - # 985 - # 990
- - ATT GAA GTC AAA AGT TGC. . . - #A ACG GAA TGG TGT TGT
3264
Leu Arg Thr Thr Thr Ala Ser Gly Lys Leu Il - #e Thr Glu Trp Cys Cys
1075 - # 1080 - # 1085
- - CGA TCT TGC ACA CTA CCA. . . - # 40 - # 45
- - Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Il - #e Pro Pro Thr Ala Gly
50 - # 55 - # 60
- - Ile Leu Lys Arg Trp Gly. . . - # 120 - # 125
- - Ser Arg Gln Glu Lys Gly Lys Ser Leu Leu Ph - #e Lys Thr Lys Asp Gly
130 - # 135 - # 140
- - Thr Asn Met Cys Thr Leu. . . - # 600 - # 605
- - Asp Gly Ser Pro Cys Lys Ile Pro Phe Glu Il - #e Met Asp Leu Glu Lys
610 - # 615 - # 620
- - Arg His Val Leu Gly Arg. . . - # 775 - # 780
- - Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Ph - #e Val Thr Asp Asn Val
785 7 - #90 7 - #95 8 -
#00
- - His Thr Trp Thr. . . - # 970 - # 975
- - Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Il - #e Glu Lys Ala Ser Phe
980 - # 985 - # 990
- - Ile Glu Val Lys Ser Cys. . . - # 1065 - # 1070
- - Leu Arg Thr Thr Thr Ala Ser Gly Lys Leu Il - #e Thr Glu Trp Cys Cys
1075 - # 1080 - # 1085
- - Arg Ser Cys Thr Leu Pro. . .
DETD . . . (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- - (ii) MOLECULE TYPE: peptide
- - (ix) FEATURE:
(A) NAME/KEY: Cleavage-sit - #e
#20) (B) LOCATION: (19
(D) OTHER INFORMATION: - #/note= "Signalase cleavage"
- - (ix) FEATURE:
(A) NAME/KEY: Cleavage-sit - #e
#86) (B) LOCATION: (85
(D) OTHER INFORMATION: - #/note= "Kex2p cleavage"
- - (ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..19
. . . #50 1 - #55 1 -
#60
- - Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile Il - #e Ile Gly Val Glu
Pro
165 - # 170 - # 175
- - Gly Gln Leu Lys Leu Asp Trp. . .
DETD . . . GGG ACC ATG TT - #T -
33
Met Leu Glu Lys Arg Glu Ala Gly Thr Met Ph - #e
20 - # 25
- - - - (2) INFORMATION FOR SEQ ID NO:41:
- - (i) SEQUENCE CHARACTERISTICS:
(A). . . (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:
- - Met Leu Glu Lys Arg Glu Ala Gly Thr Met Ph - #e
1 5 - # 10
- - - - (2) INFORMATION FOR SEQ ID NO:42:
- - (i) SEQUENCE CHARACTERISTICS:
. . .
CLM What is claimed is:
. . . selected from the group consisting of a strain of dengue, a strain of Japanese encephalitis virus (JEV), a strain of **yellow fever virus** (YF), and a strain of tick-borne encephalitis virus (TBE) which composition contains an adjuvant; and a portion of the envelope protein (**E**) of the Flavivirus strain against which said responses are sought, which portion is 80% **E**, wherein said 80% **E** represents that portion of the envelope protein that constitutes 80% of its length starting from amino acid 1 at its. . .
. . . selected from the group consisting of a strain of dengue, a strain of

Japanese encephalitis virus (JEV), a strain of **yellow fever virus** (YF), and a strain of tick-borne encephalitis virus (TBE) which composition contains an adjuvant; and a portion of the envelope protein (**E**) of the Flavivirus strain against which generation of said response is sought, which portion is 80% **E**, wherein said 80% **E** represents that portion of the envelope protein that constitutes 80% of its length starting from amino acid 1 at its. . .

7. The immunogenic composition of claim 4 wherein the 80% **E** is encoded in a DNA construct operably linked downstream from human tissue plasminogen activator prepropeptide secretion leader (tPA_L).

11. The immunogenic composition of claim 1 wherein the 80% **E** is encoded in a DNA construct operably linked downstream from a human tissue plasminogen activator prepropeptide secretion leader (tPA_L) sequence.

. . .

L6 ANSWER 6 OF 7 USPATFULL on STN

2000:142128 Methods of preparing carboxy-terminally truncated recombinant flavivirus envelope glycoproteins employing drosophila melanogaster expression systems.

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Nakano, Eilen, Honolulu, HI, United States
Clements, David, Honolulu, HI, United States
Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)

US 6136561 20001024

APPLICATION: US 1997-937195 19970925 (8)

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB . . . that cause significant morbidity in humans including the dengue (DEN) virus, Japanese encephalitis (JE) virus, tick-borne encephalitis virus (TBE), and **yellow fever virus** (YF). Flaviviruses are generally transmitted to vertebrates by chronically infected mosquito or tick vectors. The viral particle which is enveloped. . . by host cell membranes, comprises a single positive strand genomic RNA and the structural capsid (CA), membrane (M), and envelope (**E**) proteins. The **E** and M proteins are found on the surface of the virion where they are anchored in the membrane. Mature **E** is glycosylated and contains functional domains responsible for cell surface attachment and intraendosomal fusion activities. Problems have arisen in the art with respect to producing recombinant forms of the **E** glycoprotein that retain their native configuration and attendant properties associated therewith (i.**e.**, ability to induce neutralizing antibody responses). To date, recombinantly produced **E** glycoproteins have suffered from a number of limitations including improper glycosylation, folding, and disulfide bond formation. The claimed invention has addressed these concerns by providing secreted recombinant forms of the **E** glycoprotein that are highly immunogenic and appear to retain their native configuration. Carboxy-terminally truncated forms of **E** containing the amino terminal 395 amino acids and a suitable secretion signal sequence were generated in Drosophila melanogaster Schneider cell. . .

SUMM . . . vectors. Flaviviruses are enveloped by host cell membrane and contain the three structural proteins capsid (C), membrane (M), and envelope (**E**). The **E** and M proteins are found on the surface of the virion where they are anchored in the membrane. Mature **E** is glycosylated, whereas M is not, although its precursor, preM, is a glycoprotein. Glycoprotein **E**, the largest structural protein, contains functional domains responsible for cell surface attachment and intraendosomal fusion activities. It is also a. . .

SUMM . . . aching muscles and joints, and rash. A fraction of cases, typically in children, results in more extreme forms of infection, i.**e.**, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Without diagnosis and prompt medical intervention, the sudden onset and rapid. . .

SUMM . . . by the single, long open reading frame are contained in a polyprotein organized in the order, C (capsid), preM/M (membrane), **E** (envelope), NS1 (nonstructural), NS2a, NS2b, NS3, NS4a, NS4b, and NS5 (Chambers, T. J. et al. Ann Rev Microbiol (1990) 44:649-688).. . .

SUMM . . . 162:167-180; DEN-3, Osatomi, K. et al. Virus Genes (1988) 2:99-108; Osatomi, K. et al. Virology (1990) 176:643-647; DEN-4, Zhao, B. **E**. et al. Virology (1986) 155:77-88; Mackow, **E**. et al. Virology (1987) 159:217-228). In addition, the compete genomic sequences of other flaviviruses are known (**e**.g., YF virus: Rice et al., Science (1985) 229:726-733).

SUMM . . . molecular structural features of the envelope protein of flaviviruses are found in Halstead, S. B. Science (1988) 239:476-481; Brandt, W. **E**. J Infect Disease (1990) 162:577-583; Chambers, T. J. et al. Annual Rev Microbiol (1990) 44:649-688; Mandl, C. W. et al. Virology

(1989) 63:564-571; and Henchal, **E**. A. and J. R. Putnak, Clin Microbiol Rev (1990) 3:376-396.

SUMM Monoclonal antibodies (Mabs) directed against purified **E** of several flaviviruses DEN-2 (Henchal et al. Am J Trop Med Hyg (1985) 34:162-169, TBE (Heinz, F. X. et al.. . .

SUMM Although the primary amino acid sequence of the flavivirus **E** glycoprotein is variable (45-80% identity), all have twelve conserved cysteine residues, forming six disulfide bridges, and hydrophilicity profiles are nearly. . . studies, monoclonal antibody binding to purified proteolytic fragments, and analysis of neutralizing antibody escape mutants of Tick-Borne Encephalitis Virus, glycoprotein **E** was divided into three antigenic domains (A, B, and C) and two transmembrane segments at its carboxy-terminus. See, for example,. . .

SUMM Recombinant dengue proteins have been expressed in several systems to date (see Putnak, R. A. (1994) Modern Vaccinology, **E**. Kurstak ed., Plenum Medical, New York, pp. 231-252, for review). Most efforts using Escherichia coli have yielded poor immunogen unable. . .

SUMM Several reports have described vaccinia-flavivirus recombinants expressing envelope protein as part of a polyprotein (**e**.g. C-preM-**E**-NS1; [Dengue] Zhao, B. G. et al. J Virol (1987) 61:4019-4022; Deubel, V. et al. J Gen Virol (1988) 69:1921-1929; Bray,. . . et al. J Virol (1991) 63:2853-2856; [YF] Hahn, Y. S. et al. Arch Virol (1990) 115:251-265), as a single protein (**e**.g. 100% **E**; [Dengue] Bray, M. et al., J Virol (1989) 63:2853-2856), or as polypeptides (**e**.g. 79% **E**-RKG; Men, R. et al. J Virol (1991) 65:1400-1407). The most successful recombinant vaccinia viruses, those capable of inducing neutralizing antibodies and protecting mice from virus challenge, were the which were secreted **E** extracellularly or accumulated **E** on the cell surface.

SUMM . . . appear to result from reduced production of the protein. It was also found that only truncations which contained 79% of **E** or less were secreted efficiently; **E** polypeptides equal to or larger than 81% **E** were not secreted efficiently.

SUMM Men et al. (1991, supra) constructed additional C-terminal truncations between 79% **E** and 81% **E** to map the amino acids responsible for the difference in secretion and immunoreactivity with HMAF of these two truncated **E** polypeptides. The results demonstrated that 79% **E** containing the additional tripeptide sequence RKG was also secreted. Although both 59% **E** and 79% **E**-RKG were secreted, only 79% **E**-RKG was detected at the cells' surface. The recombinant Vaccinia viruses containing various truncations were also used to immunize mice. Mice immunized with recombinants expressing 79% **E**-RKG or larger portions of the envelope protein were protected. However, except for 59% **E**, mice immunized with 79% **E** or a smaller product were only partially protected. The 59% **E** elicited high protection rates (>90%) comparable to 79% **E**-RKG and larger C-terminal truncated **E** polypeptides. Protection correlated with binding to HMAF.

SUMM . . . et al. Am J Trop Med Hyg (1991) 45:159-167; Deubel, V. et al. Virology (1991) 180:442-447). Baculovirus-expressed dengue and JE **E** glycoprotein elicited neutralizing antibodies, protected mice from a lethal dengue virus challenge, or both. In spite of these successes, the. . .

SUMM . . . antibodies in mice (Srivastava, A. K. et al. Acta Virol (1990) 34:228-238). Immunization of mice with a larger polypeptide (JE **E** amino acid 319 to NS1 amino acid 65) spanning the 8 kD peptide expressed in Escherichia coli as a fusion. . .

SUMM . . . Virol (1990) 71:2107-2114 identified two domains of the DEN-1 envelope protein: domain I which includes amino acids 76-93 of the **E** protein and domain II (equivalent to domain B) which includes amino acids 293-402. These domains were identified from deletion analysis using recombinant fusion proteins expressed in **E**. coli and reacted with antiviral monoclonal antibodies. Recombinant fusion proteins containing **E**. coli trpE sequences fused to the envelope protein (amino acids 1 to 412) elicited antibodies in mice which reacted with. . .

SUMM In addition, Mason, P. W. et al. (J Gen Virol (1989) 70:2037-2049) expressed a collection of **E**. coli trpE fusion proteins to segments of JE virus envelope protein spanning domain B. The trpE fusion proteins containing the smallest JE **E** fragments that retained immunoreactivity with a panel of neutralizing monoclonal antibodies included amino -acid residues from methionine 303 through tryptophan. . .

SUMM Trirawatanapong, T. et al. Gene (1992) 116:139-150. prepared several truncated forms of dengue 2 envelope proteins in **E**. coli for epitope mapping, and mapped monoclonal antibody 3H5 to its corresponding epitope. This was first localized between amino acids. . . amino acids between positions 386 and 397. The mapping was apparently confirmed by the ability of a synthetic peptide containing **E** protein amino acids 386-397 to bind 3H5 specifically.

SUMM Megret, F. et 41. Virology (1992) 187:480-491 prepared 16 overlapping fragments of DEN-2 envelope protein as trpE fusion products in **E**. coli

for epitope mapping. The fusion proteins are produced intracellularly and obtained from the lysates. These products were used to. . .

SUMM . . . findings of Trirawatanapong et al. Gene (1992, supra), MAb 3H5 was unable to bind to trpE fusion proteins containing DEN-2 **E** amino acids 304-397, 298-385, or 366-424. The two exceptional MAbs in the findings of Megret et al. are MAbs 5A2. . .

SUMM . . . a form which mimics the native protein and is thus capable of eliciting an immune response. The only recombinantly produced **E** polypeptides containing the B domain that elicited a protective immune response in mice were expressed from Vaccinia and baculovirus vectors.. . . extensions do not interfere with the immunogenic effectiveness or secretion of the B domain. In one embodiment, such extensions are minimal--i.**e**., not more than six additional amino acids--at either the N-terminus or the C-terminus, or distributed between these termini; preferably no. . .

SUMM . . . domain includes at least portions of the region extending to amino acid 413, the additional region may confer additional functions, **e**.g., enhancing immunogenicity by providing a helper T cell epitope. The form of domain B which includes positions about 296-413 is. . .

SUMM Other portions of the **E** protein illustrated below are self-explanatory. 80% **E** is the N-terminal 80% of the protein from residue 1 to residue 395. 60% **E** represents the corresponding shorter sequence. These subunits are produced from vectors containing the DNA encoding the mature protein, or along with the **prM** fusion which results in secretion of the 80% or 60% per se.

SUMM . . . of eliciting the production of neutralizing antibodies against-dengue virus. In the illustrations below, the B domain of the envelope protein (**E**) is secreted from yeast by producing it in an expression vector containing the α-mating factor prepropeptide leader sequence (preproMFα_L). Peptide subunits representing 60% **E** and 80% **E** are secreted from Drosophila cells using the human tissue plasminogen activator secretion signal sequence for the propeptide (tPA$_L$) or from the homologous premembrane (**prM**) leader. The secreted products can easily be purified and prepared as a vaccine.

SUMM . . . virus. The vaccine contains, as active ingredient, the envelope protein of a dengue virus serotype or a subunit thereof. The **E** or subunit is secreted as a recombinantly produced protein from eucaryotic cells. The vaccine may further contain portions of additional dengue virus serotype **E** proteins similarly produced.

DRWD . . . from Mandl, et al. (supra) showing a model of the envelope protein of flaviviruses. Model of the TBE virus protein **E**. Open circles represent hydrophilic amino acid residues (Arg, Lys, Asn, Asp, Gln, Glu, His), dotted circles show intermediate amino acid. . . are not utilized. Two solid lines stand for the lipid membrane that is spanned by two transmembrane regions of protein **E**. The polypeptide chain is folded to indicate the antigenic domains A, B, and C, which are designated by large capital. . .

DRWD . . . of SEQ ID NO:1 and SEQ ID NO:4 through SEQ ID NO:7) shows the oligonucleotide used to mutagenize an 80% **E** cDNA clone to obtain the domain B coding sequence. Shown are the necleotide sequence and the corresponding translation of DEN-2 PR159/S1 **E** sequences between genomic nucleotides 1789 and 1848, the sequence of the oligonucleotide used in mutagenesis, and the resulting sequence and. . .

DETD . . . envelope protein extending approximately from the Gly at position 296 to the Gly at position 395, and optionally including additional **E** sequence through position 413 of the protein, and other portions of **E**, i.**e**., 60% **E** and 80% **E** are effectively secreted by certain convenient eucaryotic recombinant hosts, in a form that permits processing to mimic the native conformation. . .

DETD . . . a peptide which spans from approximately Gly 296 to Gly 395 of the DEN-2 envelope protein, and optionally including additional **E** sequence through position 413 of the envelope protein. These positions are approximate; for example, Mandl (1989, supra) describes the generation of a tryptic fragment containing domain B which spans the amino acids of the TBE **E** protein from position 301 to 396. The sequences described in the present application represent the envelope protein from dengue Type. . . ovary cells. Other insect cells may also be used in conjunction with baculovirus based vectors. The B domain or 60% **E** or 80% **E** must be produced as a correctly processed protein and secreted.

DETD . . . invention. An additional preferred embodiment employs Drosophila cells and the human tissue plasminogen activator leader sequence for secretion of 60% **E** or 80% **E** as well as domain B. Envelope protein subunits that represent N-terminal portions of truncated protein may also be secreted from the homologous **prM** fusion. Other secretion signal peptides or secretion leader pre/pro peptides, such as those associated with invertase or acid phosphatase of. . .

DETD The properly processed **E** protein or subunit is recovered from the cell

culture medium, purified, and formulated into vaccines. Purification and vaccine formulation employ. . .

DETD . . . can themselves be used as passive vaccines. For production of passive vaccine, a suitable mammalian subject is immunized with the **E** protein or subunit of the invention and antibodies are either recovered directly as a composition from the antisera or indirectly. . .

DETD In addition to use in vaccines or in the generation of passive vaccines, the mature recombinant **E** protein and subunits of the invention may be used as analytical reagents in assessing the presence or absence of antidengue. . .

DETD Thus, the secreted protein, such as 60% **E**, 80% **E** or B domain may be adsorbed onto solid support and the support then treated with a sample to be tested. . .

DETD In addition, both the mature peptides, such as domain B and 60% **E** or 80% **E** of the invention and the antibodies immunoreactive with it can be used in standard purification procedures as affinity reagents. Thus,. . .

DETD In the examples below, particular subunits of the dengue Type 2 envelope protein, in particular 60% **E**, 80% **E** and domain B are illustrated as representative of effective subunits of the envelope protein. For the 60% **E** and 80% **E** constructs in general, secretion can be obtained from constructions designed to express the prME subunit fusion. The mature N-terminus of. . . subunits were fused to a heterologous leader, such as the human tissue plasminogen activator leader sequence, or to the homologous **prM** sequence, the mature form of the truncated envelope protein is secreted. The secreted truncated Es are expressed at high levels. . . secreted into the medium. The products are glycosylated and processed to an endo-H resistant form. The secreted form of truncated **E** produced cotranslationally with **prM** generally represents about 20-30% of the total protein in the medium. Furthermore, based upon reactivity with conformationally sensitive monoclonal antibodies, using a ELISA and immunofluorescence formats, the secreted **E** products are shown to have a native conformation. Immunization of mice with crude medium from transformed cells expressing **prM**-truncated **E** induces a potent virus-neutralizing response.

DETD . . . wild-type sequence. There are no nucleotide differences in the Capsid and preMembrane protein-encoding portions and there are four in the **E** encoding portion.

DETD In the **E** gene, three of the four mutations are silent; S1 has G instead of A at position 1314, T rather than. . . conservative, mutagenesis studies of other viral structural proteins (Coller, B. G. et al. (1994) Mutagenesis Studies on the Predicted VP2 **E**-F Loop of Coxsackievirus B3, Abstract, 13th Annual Meeting of the American Society for Virology) have demonstrated that even relatively conservative. . .

DETD Various **E** gene subclones were obtained which represented the amino-terminal 90% of the envelope, 80% of the envelope, 60% of the envelope. . . et al. at its carboxy end which can be included in some forms of the domain B of the invention, **e**.g., DomB+T.

DETD The portion of the genome that encodes 80% of the envelope protein (80% **E**) was amplified using the Polymerase Chain Reaction, primers D2E937p and D2E2121m, and plasmid pC8 (Hahn et al. (1988, supra) as. . .

DETD In this notation of the primers, the virus serotype is first indicated (D2 for DEN-2), then the corresponding dengue gene--i.**e**., in this case envelope, **E**, is noted. Then is noted the number in the dengue cloned sequences of FIGS. 2 or 3 for the first dengue nucleotide in the 5'-3' direction of the oligonucleotide, i.**e**., using the numbering of Hahn et al. (1988, supra), and finally the notation shows whether the oligonucleotide primes the plus. . .

DETD The D2E2121m primer placed two stop codons after the 395th codon of **E**. The 80% **E** amplified cDNA fragment was digested at the XbaI sites in the cloning adapters and cloned into the NheI site of pBR322 to obtain p29D280E. Double-strand sequence for 80% **E** was determined, which identified a single silent PCR-introduced mutation at nucleotide 2001 (AAC/Asn to AAT/Asn).

DETD A subclone representing domain B was obtained from the 80% **E** subclone by oligonucleotide-directed mutagenesis. In the mutagenesis, stop codons and restriction endonuclease sites were inserted between domain C- and domain. . . FIG. 4, to avoid a high AT content in the mutagenic oligonucleotide, the stop codons defining the carboxy-terminus of 60% **E** containing domains A and C were positioned four codons upstream of the beginning of domain B, i.**e**., following Lys291. The original and altered nucleotide sequences of the mutagenized region and the corresponding amino acid translation are shown. . .

DETD To perform the mutagenesis, a 580 bp BamHI fragment spanning domain B from the pBR322-80% **E** clone p29D280E was subcloned into pGEM3Zf (Promega) to yield p29GEB2. (See FIG. 5.) This BamHI fragment encodes the 3' end. . .

DETD The cloned cDNA fragments encoding B domain and 80% **E** were inserted into expression vectors so as to maintain the translational frame of

fusions to secretion leaders as described below.. . .

DETD . . . cloning sites, and use the TRP1 gene as a selectable marker. They contain sequences derived from pBR322 to provide an **E**. coli origin of replication, the ampicillin resistance gene, and sequences derived from the 2-micron plasmid of S. cerevisiae to enable. . .

DETD . . . a fusion with the MFα_L. This construct includes those sequences that lie between domain B and the transmembrane anchor of **E**. This region contains a potential T cell epitope (Mandl et al. J Virol (1989) 63:564-571) and additional hydrophobic sequences, a. . .

DETD The domain B+stem cDNA fragment was constructed in **E**. coli cloning vectors by combining the domain B cDNA fragment and the 3' end of a 90% **E** clone. As introduced in Example 1, an **E** gene subclone representing the amino terminal 90% of **E** was constructed from DEN-2 PR159/S1 cDNA plasmid pC8 of Hahn et al. (1988, supra) using the PCR. The 90% **E** polypeptide contains all of **E** except for the C-terminal membrane anchor comprising two transmembrane domains. The 90% **E** cDNA clone was made as follows. The 90% **E** fragment was amplified by the PCR using pC8 as template and primers D2E937p and D2E2271m. The sequence of D2E937p is. . .

DETD . . . of #, and the two primers positioned useful restriction enzyme sites at both ends of the fragment. The PCR-amplified 90% **E** cDNA fragment was made blunt at both ends and cloned into the SmaI site of a modified pUC13 cloning vector,. . .

DETD Combining domain B and the 90% **E** 3' end made use of a unique AflIII restriction enzyme site found in most pUC-like cloning vectors and a unique AflIII site in domain B sequences. This combining was accomplished by first subcloning the 90% **E** fragment from pVZ90E into pBluescript to reverse the orientation of 90% **E** relative to the vector sequences, yielding pBS90E. Then, p29GEB24PS, containing domain B sequences in PGEM (Example 1), and pBS90E were. . . AflIII, and the vector-domain-B5' fragment and the domain-B3'-stem-vector fragment from the two digestions, respectively, were purified, ligated, and recovered in **E**. coli yielding pBS-Bstem.

DETD . . . flaviviruses, when assayed using a similar ELISA format. Flavivirus infected murine sera tested include, Japanese Encephalitis virus, Tick-Borne Encephalitis virus, **Yellow Fever virus**, Saint Louis Encephalitis virus, West Nile virus, three viral isolates of dengue serotype 1, two viral isolates of dengue serotype. . .

DETD To perform the sandwich enzyme immunoassay, 100 μl of anti-Dengue monoclonal antibody 9D12 or 3H5 (Henchal, **E**. A. et al., Am J Trop Med Hyg (1985) 34:162-169) was used to coat microtiter wells. The monoclonal antibodies were. . .

DETD **E**. DomB Immunizations for Hybridoma Generation:

DETD Production of 60% **E** and 80% **E** in Drosophila

DETD . . . Drosophila metalothionein gene, the human tissue plasminogen activator signal and the SV-40 early polyadenylation signal, the nucleotide sequences encoding 80% **E**, **prM** 80% **E**, 60% **E** and **prM** 60% **E** are inserted and the resulting vectors used to transform Schneider cells as described in Example 9. The mature truncated forms. . .

DETD . . . - #C CCA CCA ACA GCA GGG

192

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Il - #**e** Pro Pro Thr Ala Gly

50 - # 55 - # 60

- - ATA TTA AAA AGA TGG GGA. . . - #T AAG ACA AAG GAC GGC

432

Ser Arg Gln Glu Lys Gly Lys Ser Leu Leu Ph - #**e** Lys Thr Lys Asp Gly

130 - # 135 - # 140

- - ACG AAC ATG TGT ACC CTC. . .

DETD . . . - #A ATG GAT CTG GAA AAA

1872

Asp Gly Ser Pro Cys Lys Ile Pro Phe Glu Il - #**e** Met Asp Leu Glu Lys

610 - # 615 - # 620

- - AGA CAT GTT TTG GGC CGC. . . - #C GTC ACA GAT AAC GTG

2400

Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Ph - #**e** Val Thr Asp Asn Val

785 7 - #90 7 - #95 8 -

#00

- - CAT ACA TGG ACA. . . - #A GAG AAA GCT TCT TTC

2976

Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Il - #**e** Glu Lys Ala Ser Phe

980 - # 985 - # 990

- - ATT GAA GTC AAA AGT TGC. . . - #A ACG GAA TGG TGT TGT

3264

Leu Arg Thr Thr Thr Ala Ser Gly Lys Leu Il - #**e** Thr Glu Trp Cys Cys

1075 - # 1080 - # 1085

- - CGA TCT TGC ACA CTA CCA. . . - # 40 - # 45

- - Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Il - #**e** Pro Pro Thr Ala Gly

50 - # 55 - # 60

- - Ile Leu Lys Arg Trp Gly. . . - # 120 - # 125
- - Ser Arg Gln Glu Lys Gly Lys Ser Leu Leu Ph - #e Lys Thr Lys Asp Gly
130 - # 135 - # 140
- - Thr Asn Met Cys Thr Leu. . . - # 600 - # 605
- - Asp Gly Ser Pro Cys Lys Ile Pro Phe Glu Il - #e Met Asp Leu Glu Lys
610 - # 615 - # 620
- - Arg His Val Leu Gly Arg. . . - # 775 - # 780
- - Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Ph - #e Val Thr Asp Asn Val
785 7 - #90 7 - #95 8 -
#00
- - His Thr Trp Thr. . . - # 970 - # 975
- - Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Il - #e Glu Lys Ala Ser Phe
980 - # 985 - # 990
- - Ile Glu Val Lys Ser Cys. . . - # 1065 - # 1070
- - Leu Arg Thr Thr Thr Ala Ser Gly Lys Leu Il - #e Thr Glu Trp Cys Cys
1075 - # 1080 - # 1085
- - Arg Ser Cys Thr Leu Pro. . .
DETD . . . (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- - (ii) MOLECULE TYPE: peptide
- - (ix) FEATURE:
(A) NAME/KEY: Cleavage-sit - #e
#20) (B) LOCATION: (19
(D) OTHER INFORMATION: - #/note= "Signalase cleavage"
- - (ix) FEATURE:
(A) NAME/KEY: Cleavage-sit - #e
#86) (B) LOCATION: (85
(D) OTHER INFORMATION: - #/note= "Kex2p cleavage"
- - (ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..19
. . . #50 1 - #55 1 -
#60
- - Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile Il - #e Ile Gly Val Glu
Pro
165 - # 170 - # 175
- - Gly Gln Leu Lys Leu Asp Trp. . .
CLM What is claimed is:
1. An expression system for the recombinant production and secretion of a portion of an envelope (**E**) protein of a Flavivirus selected from the group consisting of dengue virus, Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBE) and **yellow fever virus** (YF), which expression system comprises Drosophila cells modified to contain a DNA molecule which comprises (a) a first nucleotide sequence encoding said portion of said **E** protein of the Flavivirus strain against which protection is sought, which portion is the N-terminal 80% of the protein from. . .
. . . secretory leader sequence is human tissue plasminogen activator prepropeptide secretion leader (tPA_L) and optionally includes the premembrane leader of the **E** protein.

3. A method to produce a portion of an **E** protein of a Flavivirus selected from the group consisting of dengue virus, Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBE) and **yellow fever virus** (YF), which method comprises (a) culturing the Drosophila cells of claim 1 in culture medium under conditions favorable for expression of the encoding nucleotide sequence so that the cells secrete said portion of the **E** protein of the Flavivirus strain against which protection is sought, which portion is the N-terminal 80% of the protein from residue 1 to residue 395 into the medium; and (b) recovering the portion of the **E** protein from the culture medium.

4. A method to produce a portion of an **E** protein of a Flavivirus selected from the group consisting of dengue virus, Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBE) and **yellow fever virus** (YF), which method comprises (a) culturing the Drosophila cells of claim 2 in culture medium under conditions favorable for expression of the encoding nucleotide sequence so that the cells secrete said portion of the **E** protein of the Flavivirus strain against which protection is sought, which portion is the N-terminal 80% of the protein from residue 1 to residue 395 into the medium; and (b) recovering the portion of the **E** protein from the culture medium.

5. The expression system of claim 1 wherein the N-terminal 80% of the **E** protein from residue 1 to residue 395 is dengue virus **E** protein.

6. The method of claim 3 wherein the N-terminal 80% of the **E** protein from residue 1 to residue 395 is dengue virus **E** protein.

7. The method of claim 4 wherein the N-terminal 80% of the **E** protein from residue 1 to residue 395 is dengue virus **E** protein.

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=>
=> d his

     (FILE 'HOME' ENTERED AT 15:35:18 ON 23 FEB 2004)

     FILE 'USPATFULL' ENTERED AT 15:35:57 ON 23 FEB 2004
L1          661 S YELLOW FEVER VIRUS
L2           59 S L1 AND (YELLOW FEVER VIRUS/CLM)
L3           17 S L2 AND (PRM AND E)
L4            4 S L3 AND (PRM/CLM)
L5           13 S L3 NOT L4
L6            7 S L3 AND E/CLM

=> s St. Louis encephalitis virus
        103077 ST
         41178 LOUIS
          5359 ENCEPHALITIS
         70100 VIRUS
L7         231 ST. LOUIS ENCEPHALITIS VIRUS
                 (ST(W)LOUIS(W)ENCEPHALITIS(W)VIRUS)

=> s l7 and louis/clm
            31 LOUIS/CLM
L8          16 L7 AND LOUIS/CLM

=> d l8,ti,1-16

L8   ANSWER 1 OF 16  USPATFULL on STN
TI     Diagnosis of flavivirus infection

L8   ANSWER 2 OF 16  USPATFULL on STN
TI     Genomic profiling: a rapid method for testing a complex biological
       sample for the presence of many types of organisms

L8   ANSWER 3 OF 16  USPATFULL on STN
TI     Viral vaccine production method

L8   ANSWER 4 OF 16  USPATFULL on STN
TI     Eleutherosides as adjuncts for vaccines and immune modulation

L8   ANSWER 5 OF 16  USPATFULL on STN
TI     Polynucleotides encoding flavivirus and alphavirus multivalent antigenic
       polypeptides

L8   ANSWER 6 OF 16  USPATFULL on STN
TI     Flavivirus and alphavirus recombinant antigen libraries

L8   ANSWER 7 OF 16  USPATFULL on STN
TI     Viral vaccine composition, process, and methods of use

L8   ANSWER 8 OF 16  USPATFULL on STN
TI     Chimeric flavivirus vectors

L8   ANSWER 9 OF 16  USPATFULL on STN
TI     Nucleic acid vaccines for prevention of flavivirus infection

L8   ANSWER 10 OF 16  USPATFULL on STN
TI     Novel antiviral activities primate theta defensins and mammalian
       cathelicidins

L8   ANSWER 11 OF 16  USPATFULL on STN
TI     Rapid assay for arthopod-borne disease vectors and pathogens

L8   ANSWER 12 OF 16  USPATFULL on STN
TI     Inhibition of membrane-associated viral replication

L8   ANSWER 13 OF 16  USPATFULL on STN
TI     GENOMIC PROFILING: A RAPID METHOD FOR TESTING A COMPLEX BIOLOGICAL
       SAMPLE FOR THE PRESENCE OF MANY TYPES OF ORGANISMS

L8   ANSWER 14 OF 16  USPATFULL on STN
TI     Induction of immunoglobulin class switching by inactivated viral vaccine

L8   ANSWER 15 OF 16  USPATFULL on STN
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TI Membrane based dot immunoassay and method of use

L8 ANSWER 16 OF 16 USPATFULL on STN
TI Tick cell lines

=> d l8,cbib,1-16

L8 ANSWER 1 OF 16 USPATFULL on STN
2004:21523 Diagnosis of flavivirus infection.
Monath, Thomas P., Harvard, MA, United States
Nichols, Jr., Richard D., Burlington, MA, United States
Acambis, Inc., Cambridge, MA, United States (U.S. corporation)
US 6682883 B1 20040127
APPLICATION: US 2001-910647 20010719 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 2 OF 16 USPATFULL on STN
2003:324624 Genomic profiling: a rapid method for testing a complex biological sample for the presence of many types of organisms.
Straus, Don, Cambridge, MA, UNITED STATES
US 2003228599 A1 20031211
APPLICATION: US 2003-395039 A1 20030321 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 3 OF 16 USPATFULL on STN
2003:257279 Viral vaccine production method.
Monath, Thomas P., Harvard, MA, UNITED STATES
Guirakhoo, Farshad, Melrose, MA, UNITED STATES
Arroyo, Juan, S. Weymouth, MA, UNITED STATES
US 2003180329 A1 20030925
APPLICATION: US 2003-342681 A1 20030115 (10)
PRIORITY: US 2002-348565P 20020115 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 4 OF 16 USPATFULL on STN
2003:250991 Eleutherosides as adjuncts for vaccines and immune modulation.
Bonagura, Vincent R., Englewood Cliff, NJ, UNITED STATES
DeVoti, James, Fort Salonga, NY, UNITED STATES
Lance, Herman W., Birmingham, AL, UNITED STATES
Mayhall, John M., JR., Birmingham, AL, UNITED STATES
US 2003175777 A1 20030918
APPLICATION: US 2003-357913 A1 20030204 (10)
PRIORITY: US 2002-360788P 20020301 (60)
US 2002-354397P 20020204 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 5 OF 16 USPATFULL on STN
2003:155723 Polynucleotides encoding flavivirus and alphavirus multivalent antigenic polypeptides.
Punnonen, Juha, Palo Alto, CA, United States
Bass, Steven H., Hillsborough, CA, United States
Whalen, Robert Gerald, Paris, FRANCE
Howard, Russell, Los Altos Hills, CA, United States
Stemmer, Willem P. C., Los Gatos, CA, United States
Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)
US 6576757 B1 20030610
APPLICATION: US 2000-724852 20001128 (9)
PRIORITY: US 1998-105509P 19981023 (60)
US 1998-74294P 19980211 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 6 OF 16 USPATFULL on STN
2003:142838 Flavivirus and alphavirus recombinant antigen libraries.
Punnonen, Juha, Palo Alto, CA, United States
Bass, Steven H., Hillsborough, CA, United States
Whalen, Robert Gerald, Paris, FRANCE
Howard, Russell, Los Altos Hills, CA, United States
Stemmer, Willem P. C., Los Gatos, CA, United States
Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)
US 6569435 B1 20030527
APPLICATION: US 2000-724969 20001128 (9)
PRIORITY: US 1998-105509P 19981023 (60)
US 1998-74294P 19980211 (60)

DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 7 OF 16 USPATFULL on STN
2003:134060 Viral vaccine composition, process, and methods of use.
Jira, Vic, El Monte, CA, UNITED STATES
Jirathitikal, Vichai, Chachoengsao, THAILAND
US 2003092145 A1 20030515
APPLICATION: US 2001-935344 A1 20010823 (9)
PRIORITY: US 2000-227520P 20000824 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 8 OF 16 USPATFULL on STN
2003:64652 Chimeric flavivirus vectors.
Kleanthous, Harold, Westford, MA, UNITED STATES
Miller, Charles, Medford, MA, UNITED STATES
Oros, Larisa, Boston, MA, UNITED STATES
US 2003044773 A1 20030306
APPLICATION: US 2002-160939 A1 20020531 (10)
PRIORITY: US 2001-295265P 20010601 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 9 OF 16 USPATFULL on STN
2003:30900 Nucleic acid vaccines for prevention of flavivirus infection.
Chang, Gwong-Jen J., Fort Collins, CO, UNITED STATES
US 2003022849 A1 20030130
APPLICATION: US 2001-826115 A1 20010404 (9)
PRIORITY: US 1998-87908P 19980604 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 10 OF 16 USPATFULL on STN
2003:30880 Novel antiviral activities primate theta defensins and mammalian cathelicidins.
Maury, Wendy, Coralville, IA, UNITED STATES
Stapleton, Jack, Iowa City, IA, UNITED STATES
Roller, Richard, Coralville, IA, UNITED STATES
Stinski, Mark, North Liberty, IA, UNITED STATES
McCray, Paul B., Iowa City, IA, UNITED STATES
Tack, Brian, Iowa City, IA, UNITED STATES
US 2003022829 A1 20030130
APPLICATION: US 2002-60102 A1 20020129 (10)
PRIORITY: US 2001-265270P 20010130 (60)
US 2001-309368P 20010801 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 11 OF 16 USPATFULL on STN
2002:307814 Rapid assay for arthopod-borne disease vectors and pathogens.
Dave, Kirti, Thousand Oaks, CA, UNITED STATES
Emmerich, Eva, Oxnard, CA, UNITED STATES
Ryan, Jeffrey, Haymarket, VA, UNITED STATES
Wirtz, Robert A., Atlanta, GA, UNITED STATES
US 2002172937 A1 20021121
APPLICATION: US 2000-505898 A1 20000217 (9)
PRIORITY: US 1999-120872P 19990219 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 12 OF 16 USPATFULL on STN
2002:268775 Inhibition of membrane-associated viral replication.
Block, Timothy M., Doylestown, PA, United States
Dwek, Raymond A., North Hinskey, UNITED KINGDOM
Blumberg, Baruch S., Philadelphia, PA, United States
Mehta, Anand, Landenberg, PA, United States
Platt, Frances, Long Hanborough, UNITED KINGDOM
Butters, Terry D., Garsington, UNITED KINGDOM
Zitzmann, Nicole, Odendorf, GERMANY, FEDERAL REPUBLIC OF
Synergy Pharmaceuticals, Inc., Somerset, NJ, United States (U.S. corporation)Thomas Jefferson University, Philadelphia, PA, United States (U.S. corporation)The Chancellor, Masters and Scholars of the University of Oxford, Oxford, UNITED KINGDOM (non-U.S. corporation)
US 6465487 B1 20021015
APPLICATION: US 1998-209033 19981210 (9)
PRIORITY: US 1997-69245P 19971211 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 13 OF 16 USPATFULL on STN
2002:164671 GENOMIC PROFILING: A RAPID METHOD FOR TESTING A COMPLEX BIOLOGICAL SAMPLE FOR THE PRESENCE OF MANY TYPES OF ORGANISMS.
STRAUS, DON, CAMBRIDGE, MA, UNITED STATES
US 2002086289 A1 20020704
APPLICATION: US 1999-333110 A1 19990615 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 14 OF 16 USPATFULL on STN
2001:182109 Induction of immunoglobulin class switching by inactivated viral vaccine.
Compans, Richard W., Atlanta, GA, United States
Sha, Zhiyi, Atlanta, GA, United States
US 2001031266 A1 20011018
APPLICATION: US 2000-733166 A1 20001208 (9)
PRIORITY: US 1999-169813P 19991208 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 15 OF 16 USPATFULL on STN
93:27001 Membrane based dot immunoassay and method of use.
Oprandy, John J., Rockville, MD, United States
The United States of America as represented by The Secretary of the Navy, Washington, DC, United States (U.S. government)
US 5200312 19930406
APPLICATION: US 1991-814160 19911230 (7)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 16 OF 16 USPATFULL on STN
84:25951 Tick cell lines.
Yunker, Conrad E., Hamilton, MT, United States
Cory, John C., Hamilton, MT, United States
Meibos, Harold R., Darby, MT, United States
The United States of Americas as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 4447537 19840508
APPLICATION: US 1981-227166 19810122 (6)
DOCUMENT TYPE: Utility; Granted.

=> d his

(FILE 'HOME' ENTERED AT 15:35:18 ON 23 FEB 2004)

FILE 'USPATFULL' ENTERED AT 15:35:57 ON 23 FEB 2004
L1 661 S YELLOW FEVER VIRUS
L2 59 S L1 AND (YELLOW FEVER VIRUS/CLM)
L3 17 S L2 AND (PRM AND E)
L4 4 S L3 AND (PRM/CLM)
L5 13 S L3 NOT L4
L6 7 S L3 AND E/CLM
L7 231 S ST. LOUIS ENCEPHALITIS VIRUS
L8 16 S L7 AND LOUIS/CLM

=> s l8 not l5
L9 13 L8 NOT L5

=> d l9,ti,1-13

L9 ANSWER 1 OF 13 USPATFULL on STN
TI Diagnosis of flavivirus infection

L9 ANSWER 2 OF 13 USPATFULL on STN
TI Genomic profiling: a rapid method for testing a complex biological sample for the presence of many types of organisms

L9 ANSWER 3 OF 13 USPATFULL on STN
TI Viral vaccine production method

L9 ANSWER 4 OF 13 USPATFULL on STN
TI Eleutherosides as adjuncts for vaccines and immune modulation

L9 ANSWER 5 OF 13 USPATFULL on STN
TI Viral vaccine composition, process, and methods of use

L9 ANSWER 6 OF 13 USPATFULL on STN

TI Chimeric flavivirus vectors

L9 ANSWER 7 OF 13 USPATFULL on STN
TI Novel antiviral activities primate theta defensins and mammalian cathelicidins

L9 ANSWER 8 OF 13 USPATFULL on STN
TI Rapid assay for arthopod-borne disease vectors and pathogens

L9 ANSWER 9 OF 13 USPATFULL on STN
TI Inhibition of membrane-associated viral replication

L9 ANSWER 10 OF 13 USPATFULL on STN
TI GENOMIC PROFILING: A RAPID METHOD FOR TESTING A COMPLEX BIOLOGICAL SAMPLE FOR THE PRESENCE OF MANY TYPES OF ORGANISMS

L9 ANSWER 11 OF 13 USPATFULL on STN
TI Induction of immunoglobulin class switching by inactivated viral vaccine

L9 ANSWER 12 OF 13 USPATFULL on STN
TI Membrane based dot immunoassay and method of use

L9 ANSWER 13 OF 13 USPATFULL on STN
TI Tick cell lines

=> d l9,cbib,ab,clm,1-13

L9 ANSWER 1 OF 13 USPATFULL on STN
2004:21523 Diagnosis of flavivirus infection.
Monath, Thomas P., Harvard, MA, United States
Nichols, Jr., Richard D., Burlington, MA, United States
Acambis, Inc., Cambridge, MA, United States (U.S. corporation)
US 6682883 B1 20040127
APPLICATION: US 2001-910647 20010719 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides methods and kits for use in diagnosing flavivirus infection.
CLM What is claimed is:
1. A method of diagnosing a mammal as being infected with a predetermined flavivirus by specifically detecting antibodies against said flavivirus in a biological test sample from said mammal, said method comprising the steps of: (a) providing a chimeric virus having the following characteristics: (i) the chimeric virus comprises nucleic acid sequences derived from two different viruses, one nucleic acid sequence is from said predetermined flavivirus structural genes prM-E, the other being a flavivirus that is used as the chimera backbone; (ii) the chimeric virus is capable of replicating; (iii) the chimeric virus is substantially neutralized by antibodies to said predetermined flavivirus and is not substantially neutralized by antibodies to other viruses, including the flavivirus used as the chimera backbone; (iv) the chimeric virus is attenuated compared to said predetermined flavivirus; and (v) the chimeric virus may be safely manipulated in the laboratory at a Biosafety level that is lower than that required for said predetermined flavivirus; (b) providing a biological test sample from said mammal, wherein the presence of said antibodies against said predetermined flavivirus in said sample is not known; (c) contacting said biological sample with said chimeric virus under neutralizing conditions; and (d) determining the presence or amount of infectious virus remaining following step (c), wherein neutralization of infectivity of said chimeric virus by said antibodies in said test sample is a measure of antibodies against said predetermined flavivirus that are present in said biological sample and identifies the mammal as being infected with said virus.

2. The method of claim 1, wherein step (d) involves inoculating a mammal with the sample-contacted chimeric virus, and then determining virus-induced illness or mortality in said mammal as a measure of non-neutralized infectious chimeric virus.

3. The method of claim 1, wherein step (d) involves inoculating a cell culture with the sample-contacted chimeric virus, and then determining cytopathic effects, absence of metabolic activity, absence of uptake of vital dyes, or plaque formation as a measure of non-neutralized infectious chimeric virus.

4. The method of claim 1, wherein said flavivirus backbone is that of yellow fever 17D virus.

5. The method of claim 1, wherein said predetermined flavivirus is a mosquito-borne flavivirus selected from the group consisting of Japanese encephalitis, Dengue (serotype 1, 2, 3, or 4), Yellow fever, Murray Valley encephalitis, St. **Louis** encephalitis, West Nile, Kunjin, Rocio encephalitis, and Ilheus viruses.

6. The method of claim 1, wherein said predetermined flavivirus is a tick-borne flavivirus selected from the group consisting of Central European encephalitis, Siberian encephalitis, Russian Spring-Summer encephalitis, Kyasanur Forest Disease, Omsk Hemorrhagic fever, Louping ill, Powassan, Negishi, Absettarov, Hansalova, Apoi, and Hypr viruses.

L9 ANSWER 2 OF 13 USPATFULL on STN
2003:324624 Genomic profiling: a rapid method for testing a complex biological sample for the presence of many types of organisms.
Straus, Don, Cambridge, MA, UNITED STATES
US 2003228599 A1 20031211
APPLICATION: US 2003-395039 A1 20030321 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides a method, referred to as genomic profiling, which simultaneously scans a complex biological sample for the presence of nucleic acid sequences (including genomic difference sequences, group-specific sequences, and DNA polymorphisms) that are diagnostic of numerous different types of organisms. Also included in the invention are probes, detection ensembles, and related molecules for use in the methods of the invention.
CLM What is claimed is:
1. A method for obtaining genetic information from a biological sample potentially comprising target nucleic acid molecules said method comprising the steps of: a) providing nucleic acid molecules that are (i) target nucleic acid molecules in said sample, or (ii) probes that hybridize to target nucleic acid molecules in said sample, or (iii) amplification products of (i) or (ii), or (iv) a genomic representation of (i); and b) detecting target nucleic acid molecules by contacting or comparing the nucleic acid molecules of (a) with a detection ensemble that has a minimum genomic derivation of greater than five and that comprises detection sequences that can detect target nucleic acid molecules.

2. The method of claim 1, further comprising the step of (c) identifying nucleic acid molecules detected in step (b).

3. The method of claim 1, wherein the detection ensemble has a minimum genomic derivation of greater than 11.

4. The method of claim 1, wherein the nucleic acid molecules of step (a) are not immobilized as size fractionated fragments in a matrix or on a solid support.

5. The method of claim 1, further comprising using fewer than four pairs of amplification sequences, to yield, if target nucleic acid molecules are present in the sample, amplification products.

6. The method of claim 5, wherein amplification is carried out using a single pair of amplification sequences.

7. The method of claim 1, wherein said method is used to quantify a target organism in said biological sample by in situ hybridization.

8. The method of claim 1, wherein prior to step (a), nucleic acid molecules of said sample are hybridized, simultaneously, with an ensemble of ID probes to yield the probes of step (a)(ii).

9. The method of claim 1, wherein the probes of step (a)(ii) include (i) a first region capable of hybridizing to a target nucleic acid molecule, and (ii) amplification sequences.

10. The method of claim 1, wherein said nucleic acid molecules of said sample are fixed to a solid support.

11. The method of claim 1, wherein said nucleic acid molecules of step (a) are in the liquid phase.

12. The method of claim 1, wherein at least some of the nucleic acid molecules of step (a) comprise one or more oligonucleotide tags.

13. The method of claim 1, wherein at least some of the probes of step (a)(ii) comprise: (i) two or more oligonucleotides that can be ligated to one another upon hybridization to a target nucleic acid molecule, and (ii) amplification sequences.

14. The method of claim 1, wherein said detection sequences of said detection ensemble are arrayed as spots in two dimensions or as parallel stripes on a solid support.

15. The method of claim 8, wherein said ensemble of ID probes includes probes that hybridize to at least two different nucleic acid molecules from each of at least ten different viruses, each of which belongs to a different genus.

16. The method of claim 1, wherein said biological sample is a gastrointestinal tract sample, and said genetic information is the identification of nucleic acid molecules in said sample from 6 or more of Escherichia coli, Salmonella, Shigella, Yersinia enterocolitica, Vibrio cholera, Campylobacter fecalis, Clostridium difficile, Rotavirus, Norwalk virus, Astrovirus, Adenovirus, Coronavirus, Giardia lamblia, Entamoeba histolytica, Blastocystis hominis, Cryptosporidium, Microsporidium, Necator americanus, Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis, Strongyloides stercoralis, Opsthorchis viverrini, Clonorchis sinensis, and Hymenoplepis nana.

17. The method of claim 1, wherein said biological sample is a respiratory tract sample, and said genetic information is the identification of nucleic acid molecules in said sample from 6 or more of Cornybacterium diphtheriae, Mycobacterium tuberculosis, Mycoplasma pneumoniae, Chlamydia trachomatis, Chlamydia pneumoniae, Bordetella pertussis, Legionella spp., Nocardia spp., Streptococcus pneumoniae, Haemophilus influenzae, Chlamydia psittaci, Pseudomonas aeruginosa, Staphylococcus aureus, Histoplasma capsulatum, Coccidoides immitis, Cryptococcus neoformans, Blastomyces dermatitidis, Pneumocystis carinii, Respiratory Syncytial Virus, Adenovirus, Herpes simplex virus, Influenza virus, Parainfluenza virus, and Rhinovirus.

18. The method of claim 1, wherein said biological sample is a blood sample, and said genetic information is the identification of nucleic acid molecules in said sample from 6 or more of Coagulase-negative staphylococci, Staphylococcus aureus, Viridans streptococci, Enterococcus spp., Beta-hemolytic streptococci, Streptococcus pneumoniae, Escherichia spp., Klebsiella spp., Pseudomonas spp., Enterbater spp., Proteus spp., Bacteroides spp., Clostridium spp., Pseudomonas aueruginosa, Cornybacterium spp., Plasmodium spp., Leishmania donovani, Toxoplasma spp., Microfilariae, Fungi, Histoplasma capsulatum, Coccidoides immitis, Cryptococcus neoformans, Candida spp., HIV, Herpes simplex virus, Hepatitis C virus, Hepatitis B virus, Cytomegalovirus, and Epstein-Barr virus.

19. The method of claim 1, wherein said genetic information is the identification of nucleic acid molecules in said sample from 6 or more of coxsakievirus A, Herpes simplex virus, **St. Louis encephalitis virus**, Epstein-Barr virus, myxovirus, JC virus, coxsakievirus B, togavirus, measles virus, a hepatitis virus, paramyxovirus, echovirus, bunyavirus, cytomegalovirus, varicella-zoster virus, HIV, mumps virus, equine encephalitis virus, lymphocytic choriomeningitis virus, rabies virus, and BK virus.

20. The method of claim 8, wherein at least 50% of the probes comprising said ensemble of nucleic acid probes are capable of hybridizing to pre-determined genomic difference sequences that are potentially present in said sample or in a genomic representation of said sample.

21. A kit for obtaining genetic information from a biological sample, comprising: a) a plurality of ID probes and/or SNP probes; and b) a detection ensemble comprising detection sequences that are congruent with probes of (a), wherein said detection ensemble has a minimum genomic derivation of greater than five.

22. The kit of claim 21, wherein (a) comprises more than ten different amplifiable probes.

23. The kit of claim 22, wherein (a) comprises more than fifty different amplifiable probes.

24. The kit of claim 23, wherein (a) comprises more than two hundred and fifty different amplifiable probes.

25. The kit of claim 21, wherein the detection ensemble has a minimum genomic derivation of greater than 11.

26. The kit of claim 21, wherein (a) comprises more than five families of amplifiable probes.

27. The kit of claim 21, wherein the probes of (a) are specific for at least two distinct taxa.

28. The kit of claim 27, wherein the probes of (a) are specific for at least two different species.

29. The kit of claim 27, wherein the probes of (a) are specific for at least two different genera.

30. The kit of claim 27, wherein the probes of (a) are specific for at least two different kingdoms.

31. The kit of claim 21, wherein the probes of (a) include probes that comprise: (i) two or more oligonucleotides that can be ligated to one another upon hybridization to an ID sequence of a target nucleic acid molecules, and (ii) amplification sequences.

32. The kit of claim 21, wherein the probes of (a) and/or the detection sequences of (b) are physically attached to distinct locations on a solid support.

33. The kit of claim 21, wherein at least 50% of the probes of (a) comprise genomic difference sequences from at least three different species.

34. The kit of claim 32, in which the detection sequences comprised by the detection ensemble that detect (i) members of a taxonomic group and (ii) closely related taxonomic groups are positioned adjacent to one another on said support.

35. An ensemble of ID probes that can be amplified using fewer than four pairs of amplification sequences and that comprises more than three families of ID probes and more than ten different ID probes.

36. The ensemble of claim 35, comprising more than fifty different amplifiable ID probes.

37. The ensemble of claim 36, comprising more than two hundred and fifty different amplifiable ID probes.

38. The ensemble of claim 35, comprising more than ten families of amplifiable ID probes.

39. The ensemble of claim 35, comprising more than twenty-five families of amplifiable ID probes.

40. The ensemble of claim 35, wherein more than two of said families of amplifiable probes are specific for non-overlapping taxa.

41. The ensemble of claim 35, wherein more than two of said families of amplifiable probes are specific for different species.

42. The ensemble of claim 35, wherein more than two of said families of amplifiable probes are specific for different genera.

43. The ensemble of claim 35, wherein more than two of said families of amplifiable probes are specific for different kingdoms.

44. The ensemble of claim 35, wherein the probes of (a) include probes that comprise: (i) two or more oligonucleotides that can be ligated to one another upon hybridization to an ID sequence of a target nucleic acid molecule, and (ii) amplification sequences.

45. The ensemble of claim 35, wherein at least 50% of said probes comprise genomic difference sequences from at least three different species.

46. The ensemble of claim 35, in which the detection sequences comprised by the detection ensemble that detect (i) members of a taxonomic group and (ii) closely related taxonomic groups are positioned adjacent to one another on a support.

47. A method for obtaining genetic information from a biological sample

potentially comprising target nucleic acid molecules, said method comprising the steps of: a) providing an ensemble of nucleic acid probes having a minimum genomic derivation of greater than five; b) contacting said ensemble of probes, simultaneously, with nucleic acid molecules of said sample; c) detecting hybridization between said probes and any target nucleic acid molecules of said sample; and d) identifying nucleic acid molecules detected in step (c).

48. The method of claim 13, wherein said oligonucleotides that can be ligated are SNP probes.

49. The method of claim 48, wherein at least some of said SNP probes comprise tag sequences that can hybridize to tag sequences in a detection ensemble comprising an ensemble of tag sequences congruent to said SNP probes.

50. The method of claim 48, wherein the detection ensemble has a minimum genomic derivation of greater than 20.

51. The method claim 50, wherein the detection ensemble has a minimum genomic variation of greater than 50.

52. The method of claim 1, wherein the amplification products of step (a)(iv) are generated by amplification of target nucleic acid molecules of step (a)(i) using no more than four pairs of amplification sequences.

53. The method of claim 52, wherein said amplification sequences direct the amplification of sequences lying between Alu repeats using Alu-specific primers.

54. The method of claim 52, wherein the detection ensemble of (b) comprises ID sites that are congruent to ID probes potentially amplified in step (a)(iv).

55. A kit for obtaining genetic information from a biological sample, comprising a) a plurality of nucleic acid primers that are capable of priming the amplification of DNA sequences flanked by repetitive sequences in target genomic DNA in a biological sample to yield ID probes; and b) a detection ensemble comprising detection sequences that are congruent with ID probes potentially amplified using the primers of (a), wherein said detection ensemble has a minimum genomic derivation of greater than five.

56. The kit of claim 55, wherein said detection ensemble has a minimum genomic derivation of greater than 20.

57. The kit of claim 55, wherein said repetitive sequences are human Alu repeats, and said primers are Alu-specific primers.

L9 ANSWER 3 OF 13 USPATFULL on STN
2003:257279 Viral vaccine production method.
Monath, Thomas P., Harvard, MA, UNITED STATES
Guirakhoo, Farshad, Melrose, MA, UNITED STATES
Arroyo, Juan, S. Weymouth, MA, UNITED STATES
US 2003180329 A1 20030925
APPLICATION: US 2003-342681 A1 20030115 (10)
PRIORITY: US 2002-348565P 20020115 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides methods for producing live, attenuated viral vaccines, such as flavivirus vaccines.
CLM What is claimed is:
1. A method of producing a vaccine comprising a live, attenuated virus, said method comprising the steps of: introducing a nucleic acid molecule corresponding to the genome of said virus into heteroploid cells; treating virus harvested from said cells with a nuclease; and formulating the nuclease-treated virus for administration as a vaccine.

2. The method of claim 1, wherein said virus is a flavivirus.

3. The method of claim 2, wherein said flavivirus is a yellow fever virus.

4. The method of claim 1, wherein said virus is a chimeric virus.

5. The method of claim 4, wherein said chimeric flavivirus comprises a yellow fever virus in which the nucleotide sequence encoding a prM-E protein is either deleted, truncated, or mutated so that functional

yellow fever virus prM-E protein is not expressed, and integrated into the genome of said yellow fever virus, a nucleotide sequence encoding a prM-E protein of a second, different flavivirus, so that said prM-E protein of said second flavivirus is expressed.

6. The method of claim 5, wherein said second flavivirus is a Japanese Encephalitis virus.

7. The method of claim 5, wherein said second flavivirus is a Dengue virus selected from the group consisting of Dengue types 1, 2, 3, and 4.

8. The method of claim 5, wherein said second flavivirus is selected from the group consisting of a Murray Valley Encephalitis virus, a **St. Louis Encephalitis virus**, a West Nile virus, a Tick-borne Encephalitis virus, a Hepatitis C virus, a Kunjin virus, a Powassan virus, a Kyasanur Forest Disease virus, and an Omsk Hemorrhagic Fever virus.

9. The method of claim 8, wherein said Tick-borne Encephalitis virus is a Central European Encephalitis virus or a Russian Spring-Summer Encephalitis virus.

10. The method of claim 5, wherein the nucleotide sequence encoding the prM-E protein of said second, different flavivirus replaces the nucleotide sequence encoding the prM-E protein of said yellow fever virus.

11. The method of claim 5, wherein the prM signal of said chimeric virus is that of yellow fever virus.

12. The method of claim 1, wherein said heteroploid cells are Vero cells.

13. The method of claim 1, wherein said nuclease Benzoase®.

14. The method of claim 1, further comprising concentrating said virus after treatment with said nuclease.

15. A vaccine composition prepared using the method of claim 1.

L9 ANSWER 4 OF 13 USPATFULL on STN
2003:250991 Eleutherosides as adjuncts for vaccines and immune modulation.
Bonagura, Vincent R., Englewood Cliff, NJ, UNITED STATES
DeVoti, James, Fort Salonga, NY, UNITED STATES
Lance, Herman W., Birmingham, AL, UNITED STATES
Mayhall, John M., JR., Birmingham, AL, UNITED STATES
US 2003175777 A1 20030918
APPLICATION: US 2003-357913 A1 20030204 (10)
PRIORITY: US 2002-360788P 20020301 (60)
US 2002-354397P 20020204 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Vaccines containing adjuvant comprising eleutherosides and related compounds are shown to be useful for the prevention of viral infections, bacterial infections and parasitic infections. The adjuvant compounds have been shown to modulate the expression of a wide variety or proteins involved in the immune response and inflammatory response. Exemplary eleutherosides and related compounds include eleutheroside A, eleutheroside B, eleutheroside C, eleutheroside D, eleutheroside E, eleutheroside F, and eleutheroside G, coniferylaldehyde, caffeic acid ethyl ester, chlorogenic acid, sinapinalcohol, isofraxidin, syringaresinol and 6,8-dimethoxy-7-hydroxycoumarin.
CLM What is claimed is:
1. A vaccine capable of modulating the immune system of a subject in need of said modulation comprising at least one of the compounds selected from the group consisting of an eleutheroside, coniferylaldehyde, caffeic acid ethyl ester, chlorogenic acid, sinapinalcohol, isofraxidin, syringaresinol and 6,8-dimethoxy-7-hydroxycoumarin, and where the eleutheroside is selected from the group of compounds selected from the group consisting of eleutheroside A, eleutheroside B, eleutheroside C, eleutheroside D, eleutheroside E, eleutheroside F, and eleutheroside G.

2. The vaccine of any of claim 1 wherein the modulation of the immune system prevents or treats viral infections.

3. The vaccine of claim 2, wherein the viral infection is caused by a virus selected from the group consisting of Human Immunodeficiency

virus, Varacella zoster virus, Herpes Simplex virus-1, Herpes Simplex virus-2, Cytomegliavirus, Epstein-Barn virus, Yellow Fever virus, Ebola virus, Influenza virus, Polio virus, Variola Virus, Rhinovirus, Measles, Mumps, Rubella, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Dengue, Rotavirus, Rabies, Japanese B encephalitis, Human Papillomavirus, **St. Louis encephalitis virus**, Human T lymphocyte virus-1, and Respiratory Syncytial Virus.

4. The vaccine of any of claim 1, wherein the modulation of the immune system prevents or treats bacterial infections.

5. The vaccine of claim 4, wherein the bacterial infection is caused by a bacteria selected from the group consisting of Mycobacterium sp. (such as M. tuberculosis), Vibrio sp. (such as V. Cholera), Mvcobacterium (such as M. leprae), Clostridium sp. (such as C. tetani), Bacilis sp. (such as B. anthracis), enterotoxic Escherichia sp. (such as E. coli), Hemophilus sp. (such as H. influenzae B), Helobacter sp. (such as H. pylori), Pertussis sp., Heliobacter sp., Diptheria sp., Shigella sp., Meningococcus sp., Pneumococcus sp., and Streptococcus sp.

6. The vaccine of any of claim 1, wherein the modulation of the immune system prevents or treats parasitic infections.

7. The vaccine of claim 6, wherein the parasitic infection is infection with a parasite selected from the group consisting of Plasmodium sp., Schistosoma sp., and Leishmania sp.

8. The vaccine of claim 1 where the vaccine modulates the expression of a protein and said modulation is involved in the treatment or prevention of at least one infection selected from the group consisting of viral, bacterial and parasitic.

9. The vaccine of claim 8 where the protein is at least one of the compounds selected from the group consisting of IL-10, HSP-70b, HSP-70-2, HSP-40, HSP-90, heat shock transcription factor-4, c-Fos, junB, ATF-3, TNF-Q human lymphoid transcription factor, CD14 differentiation antigen, MHC class II HLA-DR2-DW12., Lck, fibroblast growth factor receptor, platelet derived endothelial growth factor, CCR2, CCR2a. CCR2b, CCR5 and CCR6.

10. The vaccine of claim 8 where said modulation increases the expression of a protein.

11. The vaccine of claim 10 where the protein is at least one of the compounds selected from the group consisting of IL-10, HSP-70b, HSP-70-2, HSP-40, HSP-90, heat shock transcription factor-4, c-Fos, junB and ATF-3.

12. The vaccine of claim 10 wherein the protein is IL-10.

13. The vaccine of claim 10 wherein the protein is IL-10.

14. The vaccine of claim 10 wherein the protein is HSP-70b.

15. The vaccine of claim 10 wherein the protein is HSP-70-2.

16. The vaccine of claim 10 wherein the protein is HSP-40.

17. The vaccine of claim 10 wherein the protein is HSP-90.

18 The vaccine of claim 10 wherein the protein is heat shock transcription factor-4.

19 The vaccine of claim 10 wherein the protein is c-Fos.

20. The vaccine of claim 10 wherein the protein is junB.

21. The vaccine of claim 10 wherein the protein is ATF-3.

22. The vaccine of claim 8 where said modulation decreases the expression of a protein.

23. The vaccine of claim 22 where the protein is at least one of the compounds selected from the group consisting of TNF-α, human lymphoid transcription factor, CD14 differentiation antigen, MHC class II HLA-DR2-DW12., Lck, fibroblast growth factor receptor, platelet derived endothelial growth factor, CCR2, CCR2a. CCR2b, CCR5 and CCR6.

24. The vaccine of claim 22 wherein the protein is TNF-α.

25. The vaccine of claim 22 wherein the protein is human lymphoid transcription factor.

26. The vaccine of claim 22 wherein the protein is CD14 differentiation antigen.

27. The vaccine of claim 22 wherein the protein is MHC class 11 HLA-DR2-DW12.

28. The vaccine of claim 22 wherein the protein is Lck.

29. The vaccine of claim 22 wherein the protein is fibroblast growth factor receptor.

30. The vaccine of claim 22 wherein the protein is platelet derived endothelial growth factor.

31. The vaccine of claim 22 wherein the protein is CCR2.

32. The vaccine of claim 22 wherein the protein is CCR2a.

33. The vaccine of claim 22 wherein the protein is CCR2b.

34. The vaccine of claim 22 wherein the protein is CCR5.

35. The vaccine of claim 22 wherein the protein is CCR6.

L9 ANSWER 5 OF 13 USPATFULL on STN
2003:134060 Viral vaccine composition, process, and methods of use.
Jira, Vic, El Monte, CA, UNITED STATES
Jirathitikal, Vichai, Chachoengsao, THAILAND
US 2003092145 A1 20030515
APPLICATION: US 2001-935344 A1 20010823 (9)
PRIORITY: US 2000-227520P 20000824 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A composition for treating or preventing virus-induced infections is described, along with a process of producing the composition and methods of the composition's use. The composition comprises viral pathogen-infected cell or tissue, or malignantly or immunologically aberrant cells or tissues which has been reduced and/or denatured. The preferred composition is administered across a mucosal surface of an animal suffering or about suffer from infection. The composition is administered as preventive or therapeutic vaccine.

CLM What is claimed is:
1. A multivalent antiviral vaccine comprising one or more heat-inactivated viral antigens, wherein at least one viral antigen is capable in producing an immune response in a host when said vaccine is administered orally at a dose that is sufficient for preventing or treating the viral disease in said host.

2. The viral antigen of claim 1 wherein said antigen is derived from influenza virus, cytomegalovirus, avian leukosis-sarcoma virus, Rous Sarcoma virus, Mammalian C-type Murine leukemia virus, Feline leukemia virus, simian sarcoma virus, B-type Mouse mammary tumor virus, D-type virus Mason-Pfizer monkey virus, simian AIDS virus, Human T-cell leukemia virus, Simian T-cell leukemia virus, bovine leukemia virus, Human immunodeficiency virus, Simian immunodeficiency virus, Feline immunodeficiency virus, Visna/maedi virus, Equine infectious anemia virus, Caprine arthritis-encephalitis virus, spumavirus, foamy virus, endogenous retrovirus, papilloma virus, respiratory syncytial virus, poliomyelitis virus, pox virus, measles virus, arbor virus, Coxsackie virus, herpes virus, hantavirus, hepatitis virus, baculovirus, mumps virus, circovirus, arenavirus, rotavirus, Colorado Tick Fever CTF virus, Eyach virus, Langat virus, Powassan virus, Omsk hemorrhagic fever virus, Crimean-Congo hemorrhagic fever virus, Yellow fever virus, Encephalitis virus, **St. Louis Encephalitis virus**, Venezualan equine encephalitis virus, Western equine encephalitis virus, Chikungunya virus, Japanese encephalitis virus, West Nile virus, Kyasanur forest disease virus, Dengue fever virus, California encephalitis virus, adenovirus, Korean haemorrhagic fever virus, hantavirus, Argentine haemorrhagic fever virus, Junin virus, Aujeszky disease virus, Pseudorabies virus, Herpesvirus, Chikungunya virus, cowpox virus, ebolavirus, Ganjam virus, herpesvirus simiae, Lassa fever virus, Louping ill virus, Lymphocytic choriomeningitis virus, Marburg virus, Milkers nodule virus, Newcastle disease virus, Omsk haemorrhagic fever virus, Orf virus, Parvovirus, Poliovirus, Pseudorabies, Rabies virus, Rift

Valley fever virus, Russian Spring-Summer encephalitis virus, Sabia virus, vaccinia virus, vesicular stomatitis virus, Western equine encephalitis virus, or Yelllow fever virus.

3. A composition for the induction of immunity to a viral pathogen in a host in need thereof, said composition comprising reduced viral pathogen formulated as an oral pill.

4. The viral pathogen of claim 3 wherein said pathogen is selected from a group consisting of influenza virus, cytomegalovirus, avian leukosis-sarcoma virus, Rous Sarcoma virus, Mammalian C-type Murine leukemia virus, Feline leukemia virus, simian sarcoma virus, B-type Mouse mammary tumor virus, D-type virus Mason-Pfizer monkey virus, simian AIDS virus, Human T-cell leukemia virus, Simian T-cell leukemia virus, bovine leukemia virus, Human immunodeficiency virus, Simian immunodeficiency virus, Feline immunodeficiency virus, Visna/maedi virus, Equine infectious anemia virus, Caprine arthritis-encephalitis virus, spumavirus, foamy virus, endogenous retrovirus, papilloma virus, respiratory syncytial virus, poliomyelitis virus, pox virus, measles virus, arbor virus, Coxsackie virus, herpes virus, hantavirus, hepatitis virus, baculovirus, mumps virus, circovirus, arenavirus, rotavirus, Colorado Tick Fever CTF virus, Eyach virus, Langat virus, Powassan virus, Omsk hemorrhagic fever virus, Crimean-Congo hemorrhagic fever virus, Yellow fever virus, Encephalitis virus, **St. Louis Encephalitis virus**, Venezualan equine encephalitis virus, Western equine encephalitis virus, Chikungunya virus, Japanese encephalitis virus, West Nile virus, Kyasanur forest disease virus, Dengue fever virus, California encephalitis virus, adenovirus, Korean haemorrhagic fever virus, hantavirus, Argentine haemorrhagic fever virus, Junin virus, Aujeszky disease virus, Pseudorabies virus, Herpesvirus, Chikungunya virus, cowpox virus, ebolavirus, Ganjam virus, herpesvirus simiae, Lassa fever virus, Louping ill virus, Lymphocytic choriomeningitis virus, Marburg virus, Milkers nodule virus, Newcastle disease virus, Omsk haemorrhagic fever virus, Orf virus, Parvovirus, Poliovirus, Pseudorabies, Rabies virus, Rift Valley fever virus, Russian Spring-Summer encephalitis virus, Sabia virus, vaccinia virus, vesicular stomatitis virus, Western equine encephalitis virus, and Yelllow fever virus.

5. An immunogen formulated as an oral pill, wherein upon oral administration, said immunogen retains the ability to elicit an immune response in a host in need of immune response.

6. The immunogen of claim 5 is administered to the host without an immune adjuvant.

7. The immunogen of claim 5 wherein said immunogen is selected from a group consisting of immunogens derived from fungi influenza virus, cytomegalovirus, avian leukosis-sarcoma virus, Rous Sarcoma virus, Mammalian C-type Murine leukemia virus, Feline leukemia virus, simian sarcoma virus, B-type Mouse mammary tumor virus, D-type virus Mason-Pfizer monkey virus, simian AIDS virus, Human T-cell leukemia virus, Simian T-cell leukemia virus, bovine leukemia virus, Human immunodeficiency virus, Simian immunodeficiency virus, Feline immunodeficiency virus, Visna/maedi virus, Equine infectious anemia virus, Caprine arthritis-encephalitis virus, spumavirus, foamy virus, endogenous retrovirus, papilloma virus, respiratory syncytial virus, poliomyelitis virus, pox virus, measles virus, arbor virus, Coxsackie virus, herpes virus, hantavirus, hepatitis virus, baculovirus, mumps virus, circovirus, arenavirus, rotavirus, Colorado Tick Fever CTF virus, Eyach virus, Langat virus, Powassan virus, Omsk hemorrhagic fever virus, Crimean-Congo hemorrhagic fever virus, Yellow fever virus, Encephalitis virus, **St. Louis Encephalitis virus**, Venezualan equine encephalitis virus, Western equine encephalitis virus, Chikungunya virus, Japanese encephalitis virus, West Nile virus, Kyasanur forest disease virus, Dengue fever virus, California encephalitis virus, adenovirus, Korean haemorrhagic fever virus, hantavirus, Argentine haemorrhagic fever virus, Junin virus, Aujeszky disease virus, Pseudorabies virus, Herpesvirus, Chikungunya virus, cowpox virus, ebolavirus, Ganjam virus, herpesvirus simiae, Lassa fever virus, Louping ill virus, Lymphocytic choriomeningitis virus, Marburg virus, Milkers nodule virus, Newcastle disease virus, Omsk haemorrhagic fever virus, Orf virus, Parvovirus, Poliovirus, Pseudorabies, Rabies virus, Rift Valley fever virus, Russian Spring-Summer encephalitis virus, Sabia virus, vaccinia virus, vesicular stomatitis virus, Western equine encephalitis virus, and Yelllow fever virus.

8. The immune response of claim 5 wherein said immune response is directed against viral infection.

9. The oral pill of claim 5 wherein the amount of immunogen comprised in said pill is between about 0.0000001% to about 20% by weight.

10. An oral composition suitable for treating or preventing a pathogen-induced infection in a host, the composition comprising a first component comprising a denatured antigen of an infection-inducing pathogen and a second component comprising a denatured tissue from a pathogen-infected host.

11. The oral composition of claim 10 said composition further comprising calcium.

12. The oral composition of claim 11 said composition further comprising magnesium.

L9 ANSWER 6 OF 13 USPATFULL on STN
2003:64652 Chimeric flavivirus vectors.
Kleanthous, Harold, Westford, MA, UNITED STATES
Miller, Charles, Medford, MA, UNITED STATES
Oros, Larisa, Boston, MA, UNITED STATES
US 2003044773 A1 20030306
APPLICATION: US 2002-160939 A1 20020531 (10)
PRIORITY: US 2001-295265P 20010601 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides chimeric flavivirus vectors including foreign peptides inserted into the envelope proteins of the vectors and methods of using these vectors.
CLM What is claimed is:
1. A method for identifying a site in the envelope protein of a chimeric flavivirus or a genetically attenuated flavivirus that is permissive for insertion of a foreign peptide, said method comprising the steps of: (i) introducing a nucleic acid molecule encoding a foreign peptide into a gene encoding a flavivirus envelope protein; (ii) generating a flavivirus vector comprising an envelope protein encoded by said gene, wherein said envelope protein comprises said foreign peptide; and (iii) determining whether the flavivirus vector generated in step (ii) is permissive for said insertion.

2. The method of claim 1, wherein said flavivirus vector is a chimeric flavivirus vector comprising the capsid and non-structural proteins of a first flavivirus and the pre-membrane and envelope proteins of a second flavivirus.

3. The method of claim 2, wherein said first flavivirus is selected from the group consisting of Japanese encephalitis, Dengue-1, Dengue-2, Dengue-3, Dengue-4, Yellow fever, Murray Valley encephalitis, St. **Louis** encephalitis, West Nile, Kunjin, Rocio encephalitis, Ilheus, ticke-borne encephalitis, Central European encephalitis, Siberian encephalitis, Russian Spring-Summer encephalitis, Kyasanur Forest Disease, Omsk Hemorrhagic fever, Louping ill, Powassan, Negishi, Absettarov, Hansalova, Apoi, and Hypr viruses.

4. The method of claim 3, wherein said first flavivirus is Japanese Encephalitis virus.

5. The method of claim 2, wherein said second flavivirus is selected from the group consisting of Japanese encephalitis, Dengue-1, Dengue-2, Dengue-3, Dengue-4, Yellow fever, Murray Valley encephalitis, St. **Louis** encephalitis, West Nile, Kunjin, Rocio encephalitis, Ilheus, ticke-borne encephalitis, Central European encephalitis, Siberian encephalitis, Russian Spring-Summer encephalitis, Kyasanur Forest Disease, Omsk Hemorrhagic fever, Louping ill, Powassan, Negishi, Absettarov, Hansalova, Apoi, and Hypr viruses.

6. The method of claim 5, wherein said second flavivirus is Yellow Fever virus.

7. The method of claim 1, wherein said foreign peptide comprises an epitope derived from an antigen of a viral, bacterial, or parasitic pathogen.

8. The method of claim 1, wherein said foreign peptide comprises an epitope derived from a tumor-associated antigen.

9. The method of claim 1, wherein said nucleic acid molecule is introduced into said envelope gene randomly by transposon mutagenesis.

10. The method of claim 1, wherein determination of whether said flavivirus vector generated in step (ii) is permissive for said insertion is carried out by analysis of (a) the infectivity of said flavivirus vector, (b) the stability of the sequence of the foreign protein upon multiple passages of the vector, (c) the growth properties of said flavivirus vector, or (d) whether the flavivirus vector can be neutralized with antibodies against the envelope protein of said first flavivirus.

11. The method of claim 10, further comprising comparing the analysis of the flavivirus vector with a similar analysis of the flavivirus from which it was derived.

12. The method of claim 1, wherein said genetically attenuated flavivirus is Yellow Fever YF 17D.

13. A flavivirus vector comprising an envelope protein that comprises a foreign peptide.

14. The flavivirus vector of claim 13, wherein said vector is a chimeric flavivirus comprising the prM and E proteins of a first flavivirus and the C and non-structural proteins of a second flavivirus.

15. The flavivirus vector of claim 14, wherein said first flavivirus is selected from the group consisting of Japanese encephalitis, Dengue-1, Dengue-2, Dengue-3, Dengue-4, Yellow fever, Murray Valley encephalitis, St. **Louis** encephalitis, West Nile, Kunjin, Rocio encephalitis, Ilheus, ticke-borne encephalitis, Central European encephalitis, Siberian encephalitis, Russian Spring-Summer encephalitis, Kyasanur Forest Disease, Omsk Hemorrhagic fever, Louping ill, Powassan, Negishi, Absettarov, Hansalova, Apoi, and Hypr viruses.

16. The flavivirus vector of claim 14, wherein said first flavivirus is Japanese Encephalitis virus.

17. The flavivirus vector of claim 14, wherein said second flavivirus is selected from the group consisting of Japanese encephalitis, Dengue-1, Dengue-2, Dengue-3, Dengue-4, Yellow fever, Murray Valley encephalitis, St. **Louis** encephalitis, West Nile, Kunjin, Rocio encephalitis, Ilheus, ticke-borne encephalitis, Central European encephalitis, Siberian encephalitis, Russian Spring-Summer encephalitis, Kyasanur Forest Disease, Omsk Hemorrhagic fever, Louping ill, Powassan, Negishi, Absettarov, Hansalova, Apoi, and Hypr viruses.

18. The flavivirus vector of claim 17, wherein said second flavivirus is Yellow Fever virus.

19. The flavivirus vector of claim 13, wherein said foreign peptide comprises an epitope derived from an antigen of a viral, bacterial, or parasitic pathogen.

20. The flavivirus vector of claim 13, wherein said foreign peptide comprises an epitope derived from a tumor-associated antigen.

21. The flavivirus vector of claim 13, wherein said vector comprises a genetically attenuated flavivirus.

22. The flavivirus vector of claim 21, wherein said genetically attenuated flavivirus is Yellow Fever YF 17D.

23. A pharmaceutical composition comprising the flavivirus vector of claim 13 and a pharmaceutically acceptable carrier or diluent.

24. A method of delivering a peptide to a patient, said method comprising administering to said patient the composition of claim 23.

25. The method of claim 24, wherein the peptide is an antigen and said administration is carried out to induce an immune response to a pathogen or tumor from which said antigen is derived.

26. A nucleic acid molecule comprising the genome of the flavivirus of claim 1 or the complement thereof.

L9 ANSWER 7 OF 13 USPATFULL on STN
2003:30880 Novel antiviral activities primate theta defensins and mammalian cathelicidins.
Maury, Wendy, Coralville, IA, UNITED STATES

Stapleton, Jack, Iowa City, IA, UNITED STATES
Roller, Richard, Coralville, IA, UNITED STATES
Stinski, Mark, North Liberty, IA, UNITED STATES
McCray, Paul B., Iowa City, IA, UNITED STATES
Tack, Brian, Iowa City, IA, UNITED STATES
US 2003022829 A1 20030130
APPLICATION: US 2002-60102 A1 20020129 (10)
PRIORITY: US 2001-265270P 20010130 (60)
US 2001-309368P 20010801 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to the use of anti-viral peptides in the inhibition and treatment of viral infections, in particular infections caused by enveloped viruses. These anti-viral peptides, some natural and others artificial, adopt either amphiphilic alpha-helical or a theta structure where the homodimeric or heterodimer peptides are joined by both cysteine bonds and circularization of the peptides. These agents may be used alone or in combination with more traditional anti-viral pharmaceuticals.

CLM What is claimed is:

1. A method for reducing the infectivity of a virus comprising contacting said virus with a first anti-viral peptide, said peptide comprising a theta defensin peptide or amphipathic alpha helical structure in a lipid environment.

2. The method of claim 1, wherein said first anti-viral peptide is a naturally-occurring peptide.

3. The method of claim 2, wherein said naturally-occurring peptide is a cathelicidin.

4. The method of claim 3, wherein said cathelicidin is selected from the group consisting of a mouse cathelicidin, a monkey cathelicidin, a human cathelicidin, and a sheep cathelicidin.

5. The method of claim 1, wherein said first anti-viral peptide is a non-naturally occurring peptide.

6. The method of claim 1, wherein said peptide is about 13 to about 35 residues in length.

7. The method of claim 5, wherein said peptide contains a non-naturally occurring amino acid.

8. The method of claim 1, wherein the virus is an enveloped virus.

9. The method of claim 1, wherein the virus infects humans and is selected from the group consisting of HIV, HSV-1, HSV-2, EBV, varicella zoster virus, CMV, herpesvirus B, HHV6, HHV8, respiratory syncytial virus (RSV), influenza A, B and C viruses, hepatitis A, hepatitis B, hepatitis C, hepatitis G, smallpox, vaccinia virus, Marburg virus, ebola virus, dengue virus, West Nile virus, hantavirus, measles virus, mumps virus, rubella virus, rabies virus, yellow fever virus, Japanese encephalitis virus, Murray Valley encephalitis virus, Rocio virus, tick-borne encephalitis virus, **St. Louis encephalitis virus**, chikungynya virus, o'nyong-nyong virus, Ross River virus, Mayaro virus, human coronaviruses 229-E and OC43, vesicular stomatitis virus, sandfly fever virus, Rift Valley River virus, Lasa virus, lymphocytic choriomeningitis virus, Machupo virus, Junin virus, HTLV-I and -II.

10. The method of claim 1, wherein the virus infects sheep and is selected from the group consisting of border disease virus, Maedi virus, and visna virus.

11. The method of claim 1, wherein the virus infects cattle and is selected from the group consisting of bovine leukemia virus, bovine diarrhea virus, bovine lentivirus, and infectious bovine rhinotracheitis virus.

12. The method of claim 1, wherein the virus infects swine and is selected from the group consisting of swinepox, African swine fever virus, hemagluttinating virus of swine, hog cholera virus, and pseudorabies virus.

13. The method of claim 1, wherein the virus infects horses and is selected from the group consisting of bovine leukemia virus, bovine diarrhea virus, bovine lentivirus, and infectious bovine rhinotracheitis virus.

14. The method of claim 1, wherein the virus infects cats and is selected from the group consisting of feline immunodeficiency virus, feline leukemia virus, and feline infectious peritonitis virus.

15. The method of claim 1, wherein the virus infects fowl and is selected from the group consisting of Marek's disease virus, turkey bluecomb virus, infectious bronchitis virus of fowl, avian reticuloendotheliosis, sarcoma and leukemia viruses.

16. The method of claim 2, wherein the naturally-occurring peptide is selected from the group consisting of SEQ ID NOS: 1, 2, 3, 4, 5, 6 and 7.

17. The method of claim 5, wherein the non-naturally-occurring peptide is selected from the group consisting of SEQ ID NOS: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24.

18. The method of claim 1, further comprising contacting said virus with a second anti-viral agent.

19. The method of claim 18, wherein said second anti-viral agent is a second anti-viral peptide distinct from said first anti-viral peptide.

20. The method of claim 18, wherein said second anti-viral agent is non-peptide pharmaceutical agent.

21. The method of claim 20, wherein said non-peptide pharmaceutical agent is selected from the group consisting of a protease inhibitor, a nucleoside analog, a viral polymerase inhibitor, and a viral integrase inhibitor.

22. The method of claim 1, wherein said first anti-viral peptide is contacted with said virus at a concentration of about 0.1 to about 50 μg per ml.

23. The method of claim 22, wherein said first anti-viral peptide is contacted with said virus at a concentration of about 1 to about 25 μg per ml.

24. The method of claim 23, wherein said first anti-viral peptide is contacted with said virus at a concentration of about 3 to about 10 μg per ml.

25. The method of claim 1, wherein said virus is located in a tissue or fluid sample.

26. The method of claim 25, wherein said tissue or fluid sample is selected from the group of whole blood, platelets, plasma, and packed blood cells.

27. The method of claim 1, wherein said virus is located in a living subject.

28. The method of claim 27, wherein said first anti-viral peptide is administered topically.

29. The method of claim 27, wherein said first anti-viral peptide is administered to a body cavity.

30. The method of claim 27, wherein said first anti-viral peptide is administered to a mucosal membrane.

31. The method of claim 27, wherein said first anti-viral peptide is administered by injection.

32. The method of claim 27, wherein said first anti-viral peptide is administered by inhalation.

33. The method of claim 27, wherein said first anti-viral peptide is administered orally.

34. The method of claim 27, wherein said first anti-viral peptide is administered to a wound site.

35. The method of claim 27, wherein said patient is immunosuppressed.

36. The method of claim 27, wherein said subject is not infected with said virus, and first anti-viral peptide is administered prior to the

virus contacting the subject.

37. The method of claim 27, wherein said first anti-viral peptide is administered subsequent to the virus contacting the subject.

38. The method of claim 37, wherein said subject is chronically infected with said virus.

39. The method of claim 37, wherein said subject is latently infected with said virus.

40. The method of claim 37, wherein said subject is acutely infected with said virus.

41. An anti-viral composition comprising a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure or a theta defensin peptide in a lipid environment, and a second anti-viral agent.

42. The composition of claim 41, wherein said second anti-viral agent is a second anti-viral peptide distinct from said first anti-viral peptide.

43. The composition of claim 41, wherein said second anti-viral agent is a non-peptide pharmaceutical agent.

44. The composition of claim 43, wherein said non-peptide pharmaceutical agent is selected from the group consisting of a protease inhibitor, a nucleoside analog, a viral polymerase inhibitor, and a viral integrase inhibitor.

45. The composition of claim 41, formulated for topical administration.

46. The composition of claim 41, formulated for inhalation.

47. The composition of claim 41, formulated for administration to a mucosal membrane.

48. The composition of claim 41, wherein said composition is located in a sterile i.v. bag.

49. The composition of claim 41, wherein said composition is located in a sterile syringe.

50. The composition of claim 41, wherein said composition is located in sterile tubing.

51. An anti-viral composition comprising a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide, and a contraceptive agent.

52. The composition of claim 51, wherein said composition is located in a condom.

53. The composition of claim 51, wherein said composition is formulated for use in a diaphragm.

54. The composition of claim 51, wherein said composition is formulated for intra-vaginal administration.

55. The composition of claim 51, wherein said contraceptive agent is spermicidal agent or a sperm anti-motility agent.

56. A method of rendering a virus-contaminated tissue or fluid sample safe for use comprising contacting said fluid sample with a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

57. A method for reducing the number of infectious virus particles in a population of viruses comprising contacting said virus population with a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

58. A method of protecting a subject from viral infection comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

59. A method for treating a subject with a viral infection comprising

administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

60. A method for preventing a recurrent viral infection in a subject harboring a latent virus comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

61. A method for controlling virus spread within a virally-infected subject comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

62. A method for reducing viral burden in a virally-infected subject comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

63. A method for reducing virus shed from a virally-infected subject comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

64. A method for reducing the percentage of virally-infected subjects in a population comprising administering to said population, regardless of viral infection status, a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

65. A method of inducing latency in a virally-infected subject comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

66. The method of claim 1, wherein said first anti-viral peptide is encoded by a nucleic acid that is contained in an expression construct under the control of a promoter active in eukaryotic cells, wherein said expression construct is delivered into a host cell, and said cell supports production and secretion of said first anti-viral peptide which contacts said virus.

67. The method of claim 66, wherein said expression construct is an adenovirus.

68. The method of claim 66, wherein said host cell is infected by said virus.

69. The method of claim 66, wherein said nucleic acid further encodes an intracellular targeting signal fused to said first anti-viral peptide.

70. The method of claim 69, wherein said intracellular targeting signal targets said peptide to one or more of the endoplasmic reticulum, the Golgi apparatus and/or the cell surface.

L9 ANSWER 8 OF 13 USPATFULL on STN
2002:307814 Rapid assay for arthopod-borne disease vectors and pathogens.
Dave, Kirti, Thousand Oaks, CA, UNITED STATES
Emmerich, Eva, Oxnard, CA, UNITED STATES
Ryan, Jeffrey, Haymarket, VA, UNITED STATES
Wirtz, Robert A., Atlanta, GA, UNITED STATES
US 2002172937 A1 20021121
APPLICATION: US 2000-505898 A1 20000217 (9)
PRIORITY: US 1999-120872P 19990219 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method is described for detecting arthropod-borne human pathogens in arthropods using a rapid dipstick format where the signal for one or more pathogens can be visually detected on a single test dipstick. Specific examples of pathogens detected include Plasmodium species, togaviruses, flaviviruses, encephalitis viruses and Ross River virus. These tests are rapid, easy to use and are suited for use in the field.
CLM What is claimed is:
1. A method for analyzing an arthropod sample for the presence of one or more analytes associated with the pathogen that causes human malaria, comprising: a) contacting a liquid permeable support with the arthropod sample and one or more detectable analyte-specific reagents that bind specifically to a protein analyte associated with a Plasmodium

sporozoite, if present, to form analyte-reagent complexes, said support comprising at least one detection area, said area having an analyte-specific capture reagent immobilized therein, said capture reagent specific for the protein analyte associated with the Plasmodium sporozoite, said capture reagent being adapted for capturing the analyte-reagent complexes; and b) detecting the presence of the detectable analyte-specific reagent in the detection area, indicating the presence of the analyte in the sample.

2. The method of claim 1, wherein the detectable analyte-specific reagent further comprises a detectable moiety selected from the group consisting of a colored moiety, a magnetic moiety, a radioactive moiety and an enzyme.

3. The method of claim 1, wherein the detectable analyte-specific reagent is deposited on the support prior to contacting the sample.

4. The method of claim 1, wherein at least three detectable analyte-specific reagents for at least three different arthropod-carried agents associated with human malaria are employed and the support comprises at least three capture reagents immobilized onto at least three different detection areas.

5. The method of claim 1, wherein the arthropod is a mosquito.

6. The method of claim 5, wherein the sample is homogenized with a grinding solution prior to contact with said support.

7. The method of claim 1, wherein the support further comprises a control area having immobilized therein at least one reagent suitable for capturing the detectable analyte-specific reagent.

8. The method of claim 1, further employing at least two detectable analyte-specific reagents, said regents specific for a protein associated with Plasmodium falciparum circumsporozoite and a second specific for a protein associated with a Plasmodium vivax sporozoite and at least two different detection areas, one area having immobilized therein a capture reagent specific for the protein associated with Plasmodium falciparum sporozoite, and a second area having immobilized therein a capture reagent specific for the protein associated with the Plasmodium vivax sporozoite.

9. The method of claim 8, wherein the Plasmodium vivax sporozoite is Plasmodium vivax 210.

10. The method of claim 8, wherein the Plasmodium vivax sporozoite is Plasmodium vivax: 247.

11. The method of claim 1, wherein the analyte-specific reagents are monoclonal antibodies.

12. The method of claim 1, wherein the detectable analyte-specific reagents are gold-antibody conjugates.

13. The method of claim 1, wherein the detectable analyte-specific reagents are colored latex-antibody conjugates.

14. A method for analyzing an arthropod sample for the presence of at least one analyte associated with at least one type of arthropod-carried agent, wherein the arthropod-carried agent is a togavirus, comprising: a) contacting a liquid permeable support with the arthropod sample and a detectable analyte-specific reagent that binds to an analyte associated with the togavirus, if present, to form analyte-reagent complex, said support comprising a detection area, said area having an analyte-specific capture reagent immobilized therein, said capture reagent specific for the analyte associated with the togavirus, said capture reagent being adapted for capturing the analyte-reagent complex; and b) detecting the presence of the detectable analyte-specific reagent in the detection area, indicating the presence of the analyte in the sample.

15. The method of claim 14, wherein the togavirus is an encephalitis virus.

16. The method of claim 14, wherein the togavirus is a flavivirus.

17. The method of claim 16, wherein the flavivirus is Dengue.

18. The method of claim 16, wherein the flavivirus is an encephalitis

virus.

19. The method of claim 14, wherein the detectable analyte-specific reagent further comprises a detectable moiety selected from the group consisting of a colored moiety, a magnetic moiety, a radioactive moiety and an enzyme.

20. The method of claim 14, wherein the detectable analyte-specific reagent is deposited on the support prior to contacting the sample.

21. The method of claim 14, wherein three detectable analyte-specific reagents are used to detect three different encehpalitis causing viruses and the support comprises three capture reagents immobilized onto three different detection areas.

22. The method of claim 14, wherein the arthropod is a mosquito.

23. The method of claim 14, wherein the sample is homogenized with a grinding solution prior to contact with said support.

24. The method of claim 14, wherein the support further comprises a control area having immobilized therein at least one reagent suitable for capturing the detectable analyte-specific reagent.

25. The method of claim 21, wherein said three viruses are Saint **Louis** Encephalitis virus, Western Equine encephalitis virus and Eastern Equine encephalitis virus.

26. The method of claim 14, wherein the analyte specific reagents are monoclonal antibodies.

27. The method of claim 14, wherein the detectable analyte-specific reagents are gold-antibody conjugates.

28. The method of claim 14, wherein the detectable analyte-specific reagents are colored latex-antibody conjugates.

29. A method for analyzing an arthropod sample for the presence of an analyte associated with a Ross River virus arthropod-carried agent, comprising: a) contacting a liquid permeable support with the arthropod sample and a detectable analyte-specific reagent that binds to an analyte associated with Ross River virus, if present, to form analyte-reagent complex, said support comprising a detection area, said area having an analyte-specific capture reagent immobilized therein, said capture reagent specific for the analyte associated with Ross River virus, said capture reagent being adapted for capturing the analyte-reagent complex; and b) detecting the presence of the detectable analyte-specific reagent in the detection area, indicating the presence of the analyte in the sample.

30. A method for analyzing an arthropod sample for the presence of two or more analytes associated with an arthropod-carried agent, comprising: a) contacting a liquid permeable support with the arthropod sample and at least two detectable analyte-specific reagents that bind to each of the analytes, if present, to form analyte-reagent complexes, said support comprising at least two detection areas, said areas each having an analyte-specific capture reagent immobilized therein, said capture reagent being adapted for capturing one of the analyte-reagent complexes; and b) detecting the presence of the detectable analyte-specific reagent in each of the detection areas, indicating the presence of the analyte in the sample.

31. A kit for analyzing an arthropod sample for the presence or absence of at least one analyte associated with an arthropod-borne agent, comprising a liquid permeable support for contacting with said arthropod sample and at least one detectable analyte-specific reagent that forms an analyte-reagent complex with said analyte, said support comprising at least two detection areas having a capture reagent immobilized therein, said capture reagent being adapted for capturing the analyte-reagent complex.

32. The kit of claim 31, further comprising at least two detectable analyte-specific reagents for at least two different arthropod-associated agents, and wherein the support further comprises at least two capture reagents immobilized onto at least two different detection areas.

33. The kit of claim 31, further comprising at least three detectable analyte-specific reagents for at least three different

arthropod-associated agents, and wherein the support further comprises at least three capture reagents immobilized onto at least three different detection areas.

34. The kit of claim 31, wherein the kit is adapted for analyzing a sample suspected of containing mosquitoes.

35. The kit of claim 31, further comprising a grinding solution for homogenizing said sample.

36. The kit of claim 31, wherein the support further comprises a control area having immobilized therein at least one analyte for capturing uncomplexed detectable analyte-specific reagent.

37. The kit of claim 31, further comprising at least two detectable analyte-specific reagents, said regents specific for a protein associated with Plasmodium falciparum sporozoite and a second specific for a protein associated with a Plasmodium vivax sporozoite and at least two different detection areas, one area having immobilized therein a capture reagent specific for the protein associated with Plasmodium falciparum sporozoite, and a second area having immobilized therein a capture reagent specific for the protein associated with the Plasmodium vivax sporozoite.

38. The kit of claim 31, wherein the analyte-specific reagents are monoclonal antibodies.

39. The kit of claim 31, wherein the detectable analyte-specific reagents are gold-antibody conjugates.

40. The kit of claim 31, wherein the detectable analyte-specific reagents are colored latex-antibody conjugates.

41. The kit of claim 31, wherein the support further comprises at least one detectable analyte-specific reagent for an analyte associated with a togavirus and at least one detection area having immobilized therein a capture reagent specific for an analyte associated with the togavirus.

42. The kit of claim 31, further comprising a hollow plastic cassette for holding the liquid permeable support.

43. The kit of claim 42, wherein the plastic cassette is formed with an opening for receiving a filter assembly adapted to clip onto the cassette above the liquid permeable support, the kit further comprising the filter assembly with a filter membrane disposed therein for filtering the sample prior to contacting the support.

L9 ANSWER 9 OF 13 USPATFULL on STN
2002:268775 Inhibition of membrane-associated viral replication.
Block, Timothy M., Doylestown, PA, United States
Dwek, Raymond A., North Hinskey, UNITED KINGDOM
Blumberg, Baruch S., Philadelphia, PA, United States
Mehta, Anand, Landenberg, PA, United States
Platt, Frances, Long Hanborough, UNITED KINGDOM
Butters, Terry D., Garsington, UNITED KINGDOM
Zitzmann, Nicole, Odendorf, GERMANY, FEDERAL REPUBLIC OF
Synergy Pharmaceuticals, Inc., Somerset, NJ, United States (U.S. corporation)Thomas Jefferson University, Philadelphia, PA, United States (U.S. corporation)The Chancellor, Masters and Scholars of the University of Oxford, Oxford, UNITED KINGDOM (non-U.S. corporation)
US 6465487 B1 20021015
APPLICATION: US 1998-209033 19981210 (9)
PRIORITY: US 1997-69245P 19971211 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for inhibiting morphogenesis of host cell membrane-budding viruses and infections caused thereby using compounds that inhibit host cell glucosidase or glucosyltransferase enzymes. Methods for treating lipid storage diseases using compounds that inhibit glucosyl transferance enzymes.
CLM What is claimed is:
1. A method for inhibiting production of a virus belonging to the Flaviviridae family comprising contacting a mammalian cell infected by said virus with an effective amount of a 1,5-dideoxy-1,5-imino-D-glucitol derivative compound having the general formula ##STR2## wherein R^1 is selected from the group consisting of H; alkyl; alkenyl; alkoxy; acyl; aryl; aralkyl; aroyl; aralkoxy; and heterocyclic groups; and R^3, R^4 and R^5 are the same or different and

are selected from the group consisting of H; acyl; and aroyl groups; and R^6 is hydrogen, or an alkyl, alkenyl, acyl, aroyl or aralkyl group; wherein said acyl, alkyl and alkenyl groups have from 1 to 14 carbon atoms and are linear or branched, substituted or unsubstituted, and said alkenyl groups have from 1 to 6 double bonds; and wherein said aryl groups and heterocyclic groups have from 7 to 14 carbon atoms and are optionally substituted by halogen, hydroxy, $C_{1-10alkyl}$; $C_{1-10alkylene}$; $C_{1-10acyl}$ or $C_{1-10alkoxy}$; and enantiomers and stereoisomers of said compound and physiologically acceptable salts or solvates of said compound, enantiomer or stereoisomer.

2. A method according to claim 1 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with an alkyl group; wherein said alkyl group has from 7 to 14 carbon atoms and is linear or branched, and substituted or unsubstituted.

3. A method according to claim 1 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with an alkoxy group, wherein said alkoxy group has from 7 to 14 carbon atoms and is linear or branched, and substituted or unsubstituted. ##STR3##

4. A method according to claim 1 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with an alkyl, alkoxy, aralyl, aralkyl, or aralkoxy.

5. A method according to claim 1 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with a nonyl group; wherein said nonyl group is linear or branched, and substituted or unsubstituted. ##STR4##

6. A method according to any one of claims 1-5 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^3, R^4, R^5 and R^6 by a hydrogen group.

7. A method according to any one of claims 2-6 wherein at least one of R^3, R^4 and R^5 is an acyl group.

8. A method according to claim 1 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound, enantiomer, stereoisomer, or physiologically acceptable salt or solvate of said compound, enantiomer, or stereoisomer is administered to a mammal.

9. A method according to claim 8 wherein the 1,5-dideoxy-1,5-imino-D-gucitol derivative compound, enantiomer, stereoisomer, or physiologically acceptable salt or solvate of said compound, enantiomer, or stereoisomer is administered to a human.

10. A method according to claim 1 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound, enantiomer, stereoisomer, or physiologically acceptable salt or solvate of said compound, enantiomer, or stereoisomer is administered to said mammalian cell in vivo.

11. A method according to claim 1 wherein said virus is a flavivirus.

12. A method according to claim 11 wherein the flavivirus is selected from the group consisting of yellow fever virus, dengue viruses 1-4, Japanese encephalitis virus, Murray Valley encephalitis, Rocio virus, West Nile fever virus, **St. Louis encephalitis virus**, tick-borne encephalitis virus, Louping ill virus, Powassan virus, Omsk hemorrhagic fever virus and Kyasanur forest disease virus.

13. A method according to claim 11 wherein the flavivirus is selected from the group consisting of yellow fever virus, a dengue virus and Japanese encephalitis virus.

14. A method according to claim 1 wherein said virus is a pestivirus.

15. A method for inhibiting production of a virus belonging to the Flaviviridae family comprising contacting a mammalian cell infected by said virus with an effective amount of a 1,5-dideoxy-1,5-imino-D-glucitol derivative compound having the general formula ##STR5## wherein R^1 is selected from the group consisting of H, alkyl, alkenyl, alkoxy, acyl, aryl, aralkyl, aroyl, aralkoxy, and heterocyclic groups; and wherein said acyl, alkyl and alkenyl groups have from 7 to 14 carbon atoms and are linear or branched, substituted or unsubstituted; said alkenyl groups have from 1 to 6 double bonds; and said aryl groups and heterocyclic groups have from 7 to 14 carbon atoms and are optionally substituted by halogen, hydroxy, $C_{1-10alkyl}$; C_{1-10} alkylene; $C_{1-10acyl}$ or $C_{1-10alkoxy}$; and enantiomers

and stereoisomers of said compound and physiologically acceptable salts or solvates of said compound, enantiomer or stereoisomer.

16. A method according to claim 15 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with an alkyl group; wherein said alkyl group has from 7 to 14 carbon atoms and is linear or branched, and substituted or unsubstituted.

17. dA method according to claim 15 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with an alkoxy group; wherein said alkoxy group has from 7 to 14 carbon atoms and is linear or branched, and substituted or unsubstituted.

18. A method according to claim 15 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with an alkyl, alkoxy, aralkyl, or aralkoxy group.

19. A method according to claim 15 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with a nonyl group; wherein said nonyl group is linear or branched, and substituted or unsubstituted.

20. A method according to claim 15 wherein the 1,5-dideoxy-1,5-imino-D-glucitol compound, enantiomer, stereoisomer, or physiologically acceptable salt or solvate of said compound, enantiomer, or stereoisomer is administered to a human in vivo.

21. A method according to claim 15 wherein said virus is a flavivirus.

22. A method according to claim 21 wherein the flavivirus is selected from the group consisting of yellow fever virus, dengue viruses 1-4, Japanese encephalitis virus, Murray Valley encephalitis, Rocio virus, West Nile fever virus, **St. Louis encephalitis virus**, tick-borne encephalitis virus, Louping ill virus, Powassan virus, Omsk hemorrhagic fever virus and Kyasanur forest disease virus.

23. A method according to claim 21 wherein the flavivirus is selected from the group consisting of yellow fever virus, a dengue virus and Japanese encephalitis virus.

24. A method according to claim 15 wherein said virus is a pestivirus.

25. A method for inhibiting production of a Hepatitis C virus comprising contacting a mammalian cell infected by said Hepatitis C virus with an effective amount of a 1,5-dideoxy-1,5-imino-D-glucitol derivative compound having the general formula ##STR6## wherein R^1 is selected from the group consisting of H; alkyl; alkenyl; alkoxy; acyl; aryl; aralkyl; aroyl; aralkoxy; and heterocyclic groups; and R^3, R^4 and R^5 are the same or different and are selected from the group consisting of H; acyl; and aroyl groups; and R^6 is hydrogen, or an alkyl, alkenyl, acyl, aroyl or aralkyl group; wherein said acyl, alkyl and alkenyl groups have from 1 to 14 carbon atoms and are linear or branched, substituted or unsubstituted, and said alkenyl groups have from 1 to 6 double bonds; and wherein said aryl groups and heterocyclic groups have from 7 to 14 carbon atoms and are optionally substituted by halogen, hydroxy, $C_{1\text{-}10}$alkyl; $C_{1\text{-}10}$alkylene; $C_{1\text{-}10}$acyl or $C_{1\text{-}10}$alkoxy; and enantiomers and stereoisomers of said compound and physiologically acceptable salts or solvates of said compound, enantiomer or stereoisomer.

26. A method according to claim 25 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with an alkyl group; wherein said alkyl group has from 7 to 14 carbon atoms and is linear or branched, and substituted or unsubstituted.

27. A method according to claim 25 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with an alkoxy group; wherein said alkoxy group has from 7 to 14 carbon atoms and is linear or branched, and substituted or unsubstituted.

28. A method according to claim 25 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with an alkyl, alkoxy, aralyl, aralkyl, or aralkoxy.

29. A method according to claim 25 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with a nonyl group; wherein said nonyl group is linear or branched, and substituted or unsubstituted.

30. A method according to any one of claims 25-29 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^3, R^4, R^5 and R^6 by a hydrogen group.

31. A method according to any one of claims 25-29 wherein at least one of R^3, R^4 and R^5 is an acyl group.

32. A method according to claim 25 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound, enantiomer, stereoisomer, or physiologically acceptable salt or solvate of said compound, enantiomer, or stereoisomer is administered to a human.

33. A method for inhibiting production of a Hepatitis C virus comprising contacting a mammalian cell infected by said Hepatitis C virus with an effective amount of a 1,5-dideoxy-1,5-imino-D-glucitol derivative compound having the general formula ##STR7## wherein R^1 is selected from the group consisting of H, alkyl, alkenyl, alkoxy, acyl, aryl, aralkyl, aroyl, aralkoxy, and heterocyclic groups; and wherein said acyl, alkyl and alkenyl groups have from 7 to 14 carbon atoms and are linear or branched, substituted or unsubstituted; said alkenyl groups have from 1 to 6 double bonds; and said aryl groups and heterocyclic groups have from 7 to 14 carbon atoms and are optionally substituted by halogen, hydroxy, $C_{1-10alkyl}$; $C_{1-10alkylene}$; $C_{1-10acyl}$ or $C_{1-10alkoxy}$; and enantiomers and stereoisomers of said compound and physiologically acceptable salts or solvates of said compound, enantiomer or stereoisomer.

34. A method according to claim 33 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with an alkyl group; wherein said alkyl group has from 7 to 14 carbon atoms and is linear or branched, and substituted or unsubstituted.

35. A method according to claim 33 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with an alkoxy group; wherein said alkoxy group has from 7 to 14 carbon atoms and is linear or branched, and substituted or unsubstituted.

36. A method according to claim 33 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with an alkyl, alkoxy, aralkyl, or aralkoxy group.

37. A method according to claim 33 wherein the 1,5-dideoxy-1,5-imino-D-glucitol derivative compound is substituted at R^1 with a nonyl group; wherein said nonyl group is linear or branched, and substituted or unsubstituted.

38. A method according to claim 33 wherein the 1,5-dideoxy-1,5-imino-D-glucitol compound, enantiomer, stereoisomer, or physiologically acceptable salt or solvate of said compound, enantiomer, or stereoisomer is administered to a human.

L9 ANSWER 10 OF 13 USPATFULL on STN
2002:164671 GENOMIC PROFILING: A RAPID METHOD FOR TESTING A COMPLEX BIOLOGICAL SAMPLE FOR THE PRESENCE OF MANY TYPES OF ORGANISMS.
STRAUS, DON, CAMBRIDGE, MA, UNITED STATES
US 2002086289 A1 20020704
APPLICATION: US 1999-333110 A1 19990615 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides a method, referred to as genomic profiling, which simultaneously scans a complex biological sample for the presence of nucleic acid sequences (including genomic difference sequences, group-specific sequences, and DNA polymorphisms) that are diagnostic of numerous different types of organisms. Also included in the invention are probes, detection ensembles, and related molecules for use in the methods of the invention.

CLM What is claimed is:
1. A method for obtaining genetic information from a biological sample potentially comprising target nucleic acid molecules, said method comprising the steps of: a) providing nucleic acid molecules that are (i) target nucleic acid molecules in said sample, or (ii) probes that hybridize to target nucleic acid molecules in said sample, or (iii) amplification products of (i) or (ii), or (iv) a genomic representation of (i); and b) detecting target nucleic acid molecules by contacting or comparing the nucleic acid molecules of (a) with a detection ensemble that has a minimum genomic derivation of greater than five and that comprises detection sequences that can detect target nucleic acid molecules.

2. The method of claim 1, further comprising the step of (c) identifying nucleic acid molecules detected in step (b).

3. The method of claim 1, wherein the detection ensemble has a minimum genomic derivation of greater than 11.

4. The method of claim 1, wherein the nucleic acid molecules of step (a) are not immobilized as size fractionated fragments in a matrix or on a solid support.

5. The method of claim 1, further comprising using fewer than four pairs of amplification sequences, to yield, if target nucleic acid molecules are present in the sample, amplification products.

6. The method of claim 5, wherein amplification is carried out using a single pair of amplification sequences.

7. The method of claim 1, wherein said method is used to quantify a target organism in said biological sample by in situ hybridization.

8. The method of claim 1, wherein prior to step (a), nucleic acid molecules of said sample are hybridized, simultaneously, with an ensemble of ID probes to yield the probes of step (a)(ii).

9. The method of claim 1, wherein the probes of step (a)(ii) include (i) a first region capable of hybridizing to a target nucleic acid molecule, and (ii) amplification sequences.

10. The method of claim 1, wherein said nucleic acid molecules of said sample are fixed to a solid support.

11. The method of claim 1, wherein said nucleic acid molecules of step (a) are in the liquid phase.

12. The method of claim 1, wherein at least some of the nucleic acid molecules of step (a) comprise one or more oligonucleotide tags.

13. The method of claim 1, wherein at least some of the probes of step (a)(ii) comprise: (i) two or more oligonucleotides that can be ligated to one another upon hybridization to a target nucleic acid molecule, and (ii) amplification sequences.

14. The method of claim 1, wherein said detection sequences of said detection ensemble are arrayed as spots in two dimensions or as parallel stripes on a solid support.

15. The method of claim 8, wherein said ensemble of ID probes includes probes that hybridize to at least two different nucleic acid molecules from each of at least ten different viruses, each of which belongs to a different genus.

16. The method of claim 1, wherein said biological sample is a gastrointestinal tract sample, and said genetic information is the identification of nucleic acid molecules in said sample from 6 or more of Escherichia coli, Salmonella, Shigella, Yersinia enterocolitica, Vibrio cholera, Campylobacter fecalis, Clostridium difficile, Rotavirus, Norwalk virus, Astrovirus, Adenovirus, Coronavirus, Giardia lamblia, Entamoeba histolytica, Blastocystis hominis, Cryptosporidium, Microsporidium, Necator americanus, Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis, Strongyloides stercoralis, Opsthorchis viverrini, Clonorchis sinensis, and Hymenoplepis nana.

17. The method of claim 1, wherein said biological sample is a respiratory tract sample, and said genetic information is the identification of nucleic acid molecules in said sample from 6 or more of Cornybacterium diphtheriae, Mycobacterium tuberculosis, Mycoplasma pneumoniae, Chlamydia trachomatis, Chlamydia pneumoniae, Bordetella pertussis, Legionella spp., Nocardia spp., Streptococcus pneumoniae, Haemophilus influenzae, Chlamydia psittaci, Pseudomonas aeruginosa, Staphylococcus aureus, Histoplasma capsulatum, Coccidoides immitis, Cryptococcus neoformans, Blastomyces dermatitidis, Pneumocystis carinii, Respiratory Syncytial Virus, Adenovirus, Herpes simplex virus, Influenza virus, Parainfluenza virus, and Rhinovirus.

18. The method of claim 1, wherein said biological sample is a blood sample, and said genetic information is the identification of nucleic acid molecules in said sample from 6 or more of Coagulase-negative staphylococci, Staphylococcus aureus, Viridans streptococci,

Enterococcus spp., Beta-hemolytic streptococci, Streptococcus pneumoniae, Escherichia spp., Klebsiella spp., Pseudomonas spp., Enterbater spp., Proteus spp., Bacteroides spp., Clostridium spp., Pseudomonas aueruginosa, Cornybacterium spp., Plasmodium spp., Leishmania donovani, Toxoplasma spp., Microfilariae, Fungi, Histoplasma capsulatum, Coccidoides immitis, Cryptococcus neoformans, Candida spp., HIV, Herpes simplex virus, Hepatitis C virus, Hepatitis B virus, Cytomegalovirus, and Epstein-Barr virus.

19. The method of claim 1, wherein said genetic information is the identification of nucleic acid molecules in said sample from 6 or more of coxsakievirus A, Herpes simplex virus, **St. Louis encephalitis virus**, Epstein-Barr virus, myxovirus, JC virus, coxsakievirus B, togavirus, measles virus, a hepatitis virus, paramyxovirus, echovirus, bunyavirus, cytomegalovirus, varicella-zoster virus, HIV, mumps virus, equine encephalitis virus, lymphocytic choriomeningitis virus, rabies virus, and BK virus.

20. The method of claim 8, wherein at least 50% of the probes comprising said ensemble of nucleic acid probes are capable of hybridizing to pre-determined genomic difference sequences that are potentially present in said sample or in a genomic representation of said sample.

21. A kit for obtaining genetic information from a biological sample, comprising: a) a plurality of ID probes and/or SNP probes; and b) a detection ensemble comprising detection sequences that are congruent with probes of (a), wherein said detection ensemble has a minimum genomic derivation of greater than five.

22. The kit of claim 21, wherein (a) comprises more than ten different amplifiable probes.

23. The kit of claim 22, wherein (a) comprises more than fifty different amplifiable probes.

24. The kit of claim 23, wherein (a) comprises more than two hundred and fifty different amplifiable probes.

25. The kit of claim 21, wherein the detection ensemble has a minimum genomic derivation of greater than 11.

26. The kit of claim 21, wherein (a) comprises more than five families of amplifiable probes.

27. The kit of claim 21, wherein the probes of (a) are specific for at least two distinct taxa.

28. The kit of claim 27, wherein the probes of (a) are specific for at least two different species.

29. The kit of claim 27, wherein the probes of (a) are specific for at least two different genera.

30. The kit of claim 27, wherein the probes of (a) are specific for at least two different kingdoms.

31. The kit of claim 21, wherein the probes of (a) include probes that comprise: (i) two or more oligonucleotides that can be ligated to one another upon hybridization to an ID sequence of a target nucleic acid molecules, and (ii) amplification sequences.

32. The kit of claim 21, wherein the probes of (a) and/or the detection sequences of (b) are physically attached to distinct locations on a solid support.

33. The kit of claim 21, wherein at least 50% of the probes of (a) comprise genomic difference sequences from at least three different species.

34. The kit of claim 32, in which the detection sequences comprised by the detection ensemble that detect (i) members of a taxonomic group and (ii) closely related taxonomic groups are positioned adjacent to one another on said support.

35. An ensemble of ID probes that can be amplified using fewer than four pairs of amplification sequences and that comprises more than three families of ID probes and more than ten different ID probes.

36. The ensemble of claim 35, comprising more than fifty different

amplifiable ID probes.

37. The ensemble of claim 36, comprising more than two hundred and fifty different amplifiable ID probes.

38. The ensemble of claim 35, comprising more than ten families of amplifiable ID probes.

39. The ensemble of claim 35, comprising more than twenty-five families of amplifiable ID probes.

40. The ensemble of claim 35, wherein more than two of said families of amplifiable probes are specific for non-overlapping taxa.

41. The ensemble of claim 35, wherein more than two of said families of amplifiable probes are specific for different species.

42. The ensemble of claim 35, wherein more than two of said families of amplifiable probes are specific for different genera.

43. The ensemble of claim 35, wherein more than two of said families of amplifiable probes are specific for different kingdoms.

44. The ensemble of claim 35, wherein the probes of (a) include probes that comprise: (i) two or more oligonucleotides that can be ligated to one another upon hybridization to an ID sequence of a target nucleic acid molecule, and (ii) amplification sequences.

45. The ensemble of claim 35, wherein at least 50% of said probes comprise genomic difference sequences from at least three different species.

46. The ensemble of claim 35, in which the detection sequences comprised by the detection ensemble that detect (i) members of a taxonomic group and (ii) closely related taxonomic groups are positioned adjacent to one another on a support.

47. A method for obtaining genetic information from a biological sample potentially comprising target nucleic acid molecules, said method comprising the steps of: a) providing an ensemble of nucleic acid probes having a minimum genomic derivation of greater than five; b) contacting said ensemble of probes, simultaneously, with nucleic acid molecules of said sample; c) detecting hybridization between said probes and any target nucleic acid molecules of said sample; and d) identifying nucleic acid molecules detected in step (c).

48. The method of claim 13, wherein said oligonucleotides that can be ligated are SNP probes.

49. The method of claim 48, wherein at least some of said SNP probes comprise tag sequences that can hybridize to tag sequences in a detection ensemble comprising an ensemble of tag sequences congruent to said SNP probes.

50. The method of claim 48, wherein the detection ensemble has a minimum genomic derivation of greater than 20.

51. The method claim 50, wherein the detection ensemble has a minimum genomic variation of greater than 50.

52. The method of claim 1, wherein the amplification products of step (a)(iv) are generated by amplification of target nucleic acid molecules of step (a)(i) using no more than four pairs of amplification sequences.

53. The method of claim 52, wherein said amplification sequences direct the amplification of sequences lying between Alu repeats using Alu-specific primers.

54. The method of claim 52, wherein the detection ensemble of (b) comprises ID sites that are congruent to ID probes potentially amplified in step (a)(iv).

55. A kit for obtaining genetic information from a biological sample, comprising a) a plurality of nucleic acid primers that are capable of priming the amplification of DNA sequences flanked by repetitive sequences in target genomic DNA in a biological sample to yield ID probes; and b) a detection ensemble comprising detection sequences that are congruent with ID probes potentially amplified using the primers of (a), wherein said detection ensemble has a minimum genomic derivation of

greater than five.

56. The kit of claim 55, wherein said detection ensemble has a minimum genomic derivation of greater than 20.

57. The kit of claim 55, wherein said repetitive sequences are human Alu repeats, and said primers are Alu-specific primers.

L9 ANSWER 11 OF 13 USPATFULL on STN
2001:182109 Induction of immunoglobulin class switching by inactivated viral vaccine.
Compans, Richard W., Atlanta, GA, United States
Sha, Zhiyi, Atlanta, GA, United States
US 2001031266 A1 20011018
APPLICATION: US 2000-733166 A1 20001208 (9)
PRIORITY: US 1999-169813P 19991208 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present disclosure provides methods and compositions for inducing an immune response to an antigen, especially in an immunogenic composition comprising sialic acid where the antigen comprises sialic acid and wherein the immunogenic composition further comprises a sialic acid binding component, e.g., an inactivated or attenuated paramyxovirus or orthomyxovirus such as an influenza virus comprising a sialic acid binding component, e.g., a neuraminidase. The compositions comprising sialic acid and a sialic acid binding component effectively induce a humoral immune response even in a human or animal which is deficient in CD4+ T cells, due to a disease such as ARC or AIDS, and there is also an immunoglobulin class switching even in the absence of CD4+ T cells.

CLM What is claimed is:

1. A method for inducing an immune response in a human or animal wherein said human or animal has a deficiency in CD4+ T cells, said method comprising the step of administering to a human or animal deficient in T cells an immunogenic composition comprising a sialic acid binding component and at least one antigen of a target cell or target virus, whereby a humoral immune response specific for at least one antigen of the target cell or target virus is induced.

2. The method of claim 1 wherein the immune response is a humoral immune response.

3. The method of claim 1 wherein said sialic acid binding component is a hemagglutinin.

4. The method of claim 2 wherein said hemagglutinin is a viral hemagglutinin.

5. The method of claim 4 wherein said viral hemagglutinin is from an orthomyxovirus.

6. The method of claim 5 wherein said viral hemagglutinin is from influenza virus.

7. The method of claim 4 wherein said viral hemagglutinin is from a paramyxovirus.

8. The method of claim 4 wherein said viral hemagglutinin is comprised in an attenuated virus preparation.

9. The method of claim 4 wherein said viral hemagglutinin is comprised within an inactivated virus preparation.

10. The method of claim 8 wherein the virus preparation is inactivated with formalin or propiolactone.

11. The method of claim 4 wherein the at least one antigen of a target cell is from a bacterial pathogen cell.

12. The method of claim 11 wherein the bacterial pathogen cell has a sialic acid capsule and wherein said capsule in present in said immunogenic composition.

13. The method of claim 12 wherein said bacterial pathogen is Neisseria meningitidis.

14. The method of claim 12 wherein said bacterial pathogen is Eseherichia coli.

15. The method of claim 2 herein said target cell is a tumor cell.

16. The method of claim 2 wherein said target virus is an enveloped virus.

17. The method of claim 16 wherein said enveloped virus is simian immunodeficiency virus, human immunodeficiency virus, feline immunodeficiency virus, or bovine immunodeficiency virus, rabies virus, measles virus, vesicular stomatitis virus, flavivirus, alphavirus or herpes virus.

18. The method of claim 17 wherein said alphavirus is Sindbis virus, Semliki forest virus, Venezuelan equine encephalitis virus, eastern equine encephalitis virus, western equine encephalitis virus, Ross River virus, Mayaro virus, O'nyong-nyong virus or chikungunya virus.

19. The method of claim 17 wherein the flavivirus is Dengue virus, yellow fever virus, **St. Louis encephalitis virus**, Japanese encephalitis virus, Murray Valley encephalitis virus, West Nile virus, Rocio virus, tick-bome encephalitis virus, Omsk hemorrhagic fever virus, Kyasanur Forest disease virus, or Powassan virus.

20. The method of claim 9 wherein the immunogenic composition comprises an inactivated virus comprising a hemagglutinin or inactivated target cell or target virus and a carrier.

21. An immunogenic composition comprising a sialic acid binding component and an inactivated or attenuated target cell or an inactivated or attenuated target virus.

22. The immunogenic composition of claim 21 wherein said sialic acid binding component is a hemagglutinin of an orthomyxovirus or a paramyxovirus.

23. The immunogenic composition of claim 21 wherein said sialic acid binding component is comprised in an inactivated or attenuated preparation of an orthomyxovirus or paramyxovirus.

24. The immunogenic composition of claim 22 further comprising a virus like particle or an inactivated or attenuated sialic acid containing virus preparation.

25. The immunogenic composition of claim 24 wherein said virus preparation is an enveloped virus preparation.

26. The immunogenic composition of claim 25 wherein said is an inactivated tumor cell. virus preparation is a preparation of simian immunodeficiency virus, human immunodeficiency virus, feline immunodeficiency virus, or bovine immunodeficiency virus, rabies virus, measles virus, vesicular stomatitis virus, flavivirus, alphavirus or herpes virus.

27. The immunogenic composition of claim 26 wherein said alphavirus is Sindbis virus, Semliki forest virus, Venezuelan equine encephalitis virus, eastern equine encephalitis virus, western equine encephalitis virus, Ross River virus, Mayaro virus, O'nyong-nyong virus or chikungunya virus.

28. The immunogenic composition of claim 26 wherein the flavivirus is Dengue virus, yellow fever virus, **St. Louis encephalitis virus**, Japanese encephalitis virus, Murray Valley encephalitis virus, West Nile virus, Rocio virus, tick-borne encephalitis virus, Omsk hemorrhagic fever virus, Kyasanur Forest disease virus, or Powassan virus.

29. The immunogenic composition of claim 21 wherein the target cell is a tumor cell.

30. The method of claim 21 wherein the at least one antigen of a target cell is from a bacterial pathogen cell.

31. The method of claim 30 wherein the bacterial pathogen cell has a sialic acid capsule and wherein said capsule in present in said immunogenic composition.

32. The method of claim 31 wherein said bacterial pathogen is Neisseria meningitidis.

33. The method of claim 30 wherein said bacterial pathogen is Escherichia coli.

34. An immunogenic composition comprising a sialic acid binding component and at least one antigen of a target cell or target virus.

35. The immunogenic composition of claim 34 wherein the sialic acid binding component is a hemagglutinin of an orthomyxovirus or a paramyxovirus.

36. The immunogenic composition of claim 35 wherein the composition comprises inactivated or attenuated orthomyxovirus or paramyxovirus.

37. The immunogenic composition of claim 34 wherein the at least one antigen of a target cell or target virus comprises sialic acid or polymerized sialic acid.

38. The immunogenic composition of claim 37 wherein the at least one antigen of a target cell or target virus is comprised within inactivated or attenuated target cell or inactivated or attenuated target virus or virus-like particles of a target virus.

39. The immunogenic composition of claim 38 wherein the target cell is Neisseria meningitidis or Escherichia coli.

40. The immunogenic composition of claim 38 wherein the target virus is simian immunodeficiency virus, human immunodeficiency virus, feline immunodeficiency virus, or bovine immunodeficiency virus, rabies virus, measles virus, vesicular stomatitis virus, flavivirus, alphavirus or herpes virus.

41. The immunogenic composition of claim 40 wherein said alphavirus is Sindbis virus, Semliki forest virus, Venezuelan equine encephalitis virus, eastern equine encephalitis virus, western equine encephalitis virus, Ross River virus, Mayaro virus, O'nyong-nyong virus or chikungunya virus.

42. The immunogenic composition of claim 40 wherein the flavivirus is Dengue virus, yellow fever virus, **St. Louis encephalitis virus**, Japanese encephalitis virus, Murray Valley encephalitis virus, West Nile virus, Rocio virus, tick-borne encephalitis virus, Omsk hemorrhagic fever virus, Kyasanur Forest disease virus, or Powassan virus.

43. The immunogenic composition of claim wherein the target cell is a tumor cell.

44. A method for inducing an immune response in a human or animal, said method comprising the steps of administering an immunogenic composition comprising a sialic acid binding compo component and at least one antigen of a target cell or target virus, whereby a humoral immune response specific for at least one antigen of the target cell or target virus is induced.

45. The method of claim 44 wherein the immune response is a humoral immune response.

46. The method of claim 45 wherein said sialic acid binding component is a hemagglutinin.

47. The method of claim 46 wherein said hemagglutinin is a viral hemagglutinin.

48. The method of claim 47 wherein said viral hemagglutinin is from an orthomyxovirus or a paramyxovirus.

49. The method of claim 48 wherein said viral hemagglutinin is from influenza virus.

50. The method of claim 47 wherein said viral hemagglutinin is comprised in an attenuated virus preparation.

51. The method of claim 47 wherein said viral hemagglutinin is comprised within an inactivated virus preparation.

52. The method of claim 51 wherein the virus preparation is inactivated with formalin or propiolactone.

53. The method of claim 46 wherein the at least one antigen of a target cell is from a bacterial pathogen cell.

54. The method of claim 53 wherein the bacterial pathogen cell has a

sialic acid capsule and wherein said capsule in present in said immunogenic composition.

55. The method of claim 54 wherein said bacterial pathogen is Neisseria meningitidis or Escherichia coli.

56. The method of claim 46 wherein said target cell is a tumor cell.

57. The method of claim 56 wherein said target virus or is an enveloped virus.

58. The method of claim 57 wherein said enveloped virus is simian immunodeficiency virus, human immunodeficiency virus, feline immunodeficiency virus, or bovine immunodeficiency virus, rabies virus, measles virus, vesicular stomatitis virus, flavivirus, alphavirus or herpes virus.

59. The method of claim 58 wherein said alphavirus is Sindbis virus, Semliki forest virus, Venezuelan equine encephalitis virus, eastern equine encephalitis virus, western equine encephalitis virus, Ross River virus, Mayaro virus, O'nyong-nyong virus or chikungunya virus.

60. The method of claim 58 wherein the flavivirus is Dengue virus, yellow fever virus, **St. Louis encephalitis virus**, Japanese encephalitis virus, Murray Valley encephalitis virus, West Nile virus, Rocio virus, tick-borne encephalitis virus, Omsk hemorrhagic fever virus, Kyasanur Forest disease virus, or Powassan virus.

61. The method of claim 62 wherein the immunogenic composition comprises an inactivated virus comprising a hemagglutinin or inactivated target cell or target virus and a carrier.

L9 ANSWER 12 OF 13 USPATFULL on STN
93:27001 Membrane based dot immunoassay and method of use.
Oprandy, John J., Rockville, MD, United States
The United States of America as represented by The Secretary of the Navy, Washington, DC, United States (U.S. government)
US 5200312 19930406
APPLICATION: US 1991-814160 19911230 (7)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Antigens or antibodies are detected using a novel membrane based immunoassay. Known antigens or antibodies which will form complexes with antigens/antibodies to be assayed are spot filtered with pressure through a membrane. The membrane, either by itself or attached to a base material as a test strip, is incubated with a test fluid. Consequently, the resulting antibody-antigen complex is incubated directly or after an intermediate anti-antibody incubation with enzyme conjugated immunoglobulin and exposed to substrate which produces a colored insoluble product if the test target is present.
CLM What is claimed is:
1. A test strip for use in an aqueous immunobinding assay made by the method comprising: filtering an aqueous solution containing a ligand selected from the group consisting of an antigen, antibody, and antigen-antibody complex, under positive pressure through a hydrophobic polyvinylidene difluoride (PVDF) membrane to form a clearly defined spot; immobilizing said ligand by drying said membrane.

2. A test strip as described in claim 1 wherein the said ligand is an antigen.

3. A test strip as described in claim 1 wherein the said ligand is an antibody.

4. A test strip as described in claim 1 wherein the said ligand is an antigen-antibody complex.

5. A test strip as described in claim 2 wherein said antigen is eastern equine encephalomyelitis virus.

6. A test strip as described in claim 2 wherein said antigen is **St. Louis encephalitis virus**.

7. A test strip as described in claim 3 wherein said antibody is an IgG mouse monoclonal antibody to meningococcal group A polysaccharide.

8. A method for making a test strip for use in an aqueous immunobinding assay comprising: filtering an aqueous solution containing a ligand

selected from the group consisting of an antigen, antibody, and antigen-antibody complex, under positive pressure through a hydrophobid PVDF membrane to form a clearly defined spot; and immobilizing said ligand by drying said membrane.

9. An aqueous immunobinding assay method for detecting an antigen or antibody target in a test solution comprising: filtering an aqueous solution containing a ligand which specifically binds to said target wherein said ligand is selected from the group consisting of an antigen, antibody, and antigen-antibody complex, under positive pressure through a hydrophobic PVDF membrane to form a clearly defined spot; pl immobilizing said ligand by drying said membrane to form a test strip; incubating said test strip in a blocking/wetting solution comprising a blocking agent in a wetting solution, wherein the amount of said blocking agent is sufficient to block all non-specific binding sites and said wetting solution comprises a surfactant in a buffer, wherein the amount of said surfactant is sufficient to reduce the hydrophobicity of said clearly defined spot; diluting said test solution in said wetting solution and incubating said test strip therein for a time sufficient for any target present to bind to said ligand and form a reacted test strip; incubating said reacted test strip with a label conjugated to an antigen or antibody which specifically binds to said target; and measuring said label to determine if said target is present.

10. The method according to claim 9 wherein said label is conjugated to an antibody and said label is selected from the group consisting of horseradish peroxidase, urease, alkaline phosphatase, glucose oxidase, and fluorescent molecules.

11. The method according to claim 10 wherein said label is horseradish peroxidase and its substrate is selected from the group consisting of 4chloro-1-naphthol and tetramethyl benzidine.

L9 ANSWER 13 OF 13 USPATFULL on STN
84:25951 Tick cell lines.
Yunker, Conrad E., Hamilton, MT, United States
Cory, John C., Hamilton, MT, United States
Meibos, Harold R., Darby, MT, United States
The United States of Americas as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 4447537 19840508
APPLICATION: US 1981-227166 19810122 (6)
DOCUMENT TYPE: Utility; Granted.

AB Six new cell lines of Acari: Ixodidae are provided, four from embryonic tissue of Dermacentor variabilis and two from embryonic tissue of Dermacentor parumapertus; and the use of these cell lines for replicating representative microorganisms is shown.

CLM What is claimed is:
1. A continuous cell line of embryonic cells of Acari: Ixodidae of the species Dermacentor variabilis designated RML-15 and having ATCC No. CRL 8052.

2. A continuous cell line of embryonic cells of Acari: Ixodidae of the species Dermacentor variabilis designated RML-18 and having ATCC No. CRL 8055.

3. A continuous cell line of embryonic cells of Acari: Ixodidae of the species Dermacentor variabilis designated RML-19 and having ATCC No. CRL 8056.

4. A continuous cell line of embryonic cells of Acari: Ixodidae of the species Dermacentor variabilis designated RML-20 and having ATCC No. CRL 8057.

5. A continuous cell line of embryonic cells of Acari: Ixodidae of the species Dermacentor parumapertus designated RML-16 and having ATCC No. CRL 8053.

6. A continuous cell line of embryonic cells of Acari: Ixodidae of the species Dermacentor parumapertus designated RML-17 and having ATCC No. CRL 8054.

7. The cell line of claims 1, 2, 3 or 4 adapted to an incubation temperature of from about 25° C. up to about 39° C.

8. The cell line of claim 7 adapted to an incubation temperature of about 37° C.

9. The cell line of claim 5 or 6 adapted to an incubation temperature of from about 25° C. to about 30° C.

10. The cell line of claim 9 adapted to an incubation temperature of about 27° C.

11. A composition consisting essentially of the cell line of any one of claims 1 to 6 maintained in a sterile medium consisting essentially of: modified Liebovitz-15 medium with glutamine and Eagle's minimal essential medium with Hank's salts and glutamine in a ratio of about 0.5-2:1; to which have been added: about 20% fetal bovine serum inactivated at 56° C. for 1 hr; about 10% tryptose phosphate broth; and about 0.1% bovine plasma albumin-fraction V; all adjusted to a pH of about 6.8.

12. The composition of claim 11 wherein after establishment, the cell line is maintained in a culture medium in which the ratio of modified Liebovitz-15 medium with glutamine to Eagle's minimal essential medium with Hank's salts and glutamine is about 1:1.

13. A method of replicating a microorganism selected from one of the group consisting of viruses, rickettsias, and spiroplasms, comprising infecting the cell line of any one of claims 1 to 6 with said microorganism and incubating the infected culture.

14. The method of claim 13 wherein the microorganism is a virus selected from the group consisting of Chikungunya (23161), O'nyong-nyong (MP30), St. **Louis** encephalitis (798-55), Yellow fever (17D), Langat (TP21), Powassan (794), Modoc (M544), Kemerovo (R10), Colorado Tick Fever (SS18), Sawgrass (PR96406), and Cascade Virus (109231D).

15. The method of claim 13 wherein the cell line infected consists essentially of cells of the species Dermacentor variabilis.

16. The method of claim 13 wherein the microorganism is a rickettsia selected from the group consisting of R. Rickettsii (R), R. akari (Kaplan), R. typhi (Wilmington), and argasid tick isolate (113704-14).

17. The method of claim 13 wherein the microoganism is a virus selected from the group consisting of Togaviridae, Reoviridae, Rhabdoviridae, Bunyaviridae, and taxonomically unclassified Arboviruses.

18. A method of isolating temperature sensitive variants of arboviruses comprising replicating the arboviruses in the cell line of any one of claims 1 to 6 at an incubation temperature set at from about 22° C. to about 36° C. and isolating the replicated variants.

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=> d his

     (FILE 'HOME' ENTERED AT 15:35:18 ON 23 FEB 2004)

     FILE 'USPATFULL' ENTERED AT 15:35:57 ON 23 FEB 2004
L1           661 S YELLOW FEVER VIRUS
L2            59 S L1 AND (YELLOW FEVER VIRUS/CLM)
L3            17 S L2 AND (PRM AND E)
L4             4 S L3 AND (PRM/CLM)
L5            13 S L3 NOT L4
L6             7 S L3 AND E/CLM
L7           231 S ST. LOUIS ENCEPHALITIS VIRUS
L8            16 S L7 AND LOUIS/CLM
L9            13 S L8 NOT L5

=> s powassan virus
             83 POWASSAN
          70100 VIRUS
L10          52 POWASSAN VIRUS
                  (POWASSAN(W)VIRUS)

=> s l10 and powassan/clm
             10 POWASSAN/CLM
L11           6 L10 AND POWASSAN/CLM

=> s l11 not l9
L12           2 L11 NOT L9

=> d l12,cbib,ab,clm
```

L12 ANSWER 1 OF 2 USPATFULL on STN
2003:324596 Method for rapid detection and identification of viral bioagents.
Ecker, David J., Encinitas, CA, UNITED STATES
Griffey, Richard H., Vista, CA, UNITED STATES
Sampath, Rangarajan, San Diego, CA, UNITED STATES
Hofstadler, Steven A., Oceanside, CA, UNITED STATES
McNeil, John, La Jolla, CA, UNITED STATES
US 2003228571 A1 20031211
APPLICATION: US 2003-405756 A1 20030331 (10)
PRIORITY: US 2002-369405P 20020401 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Method for detecting and identifying unknown bioagents, including bacteria, viruses and the like, by a combination of nucleic acid amplification and molecular weight determination using primers which hybridize to conserved sequence regions of nucleic acids derived from a bioagent and which bracket variable sequence regions that uniquely identify the bioagent. The result is a "base composition signature" (BCS) which is then matched against a database of base composition signatures, by which the bioagent is identified.

CLM What is claimed is:

1. A method of identifying a virus strain in a sample comprising: a) contacting nucleic acid from the sample with at least one pair of oligonucleotide primers which hybridize to sequences of the nucleic acid, wherein the sequences flank a variable acid sequence of the virus strain; b) amplifying the variable nucleic acid sequence to produce an amplification product; c) determining the molecular mass or base composition of the amplification product; and d) identifying the virus strain.

2. The method of claim 1 wherein step d) comprises comparing the molecular mass or base composition of the amplification product to one or more molecular masses or base compositions of amplification products obtained by performing steps a) through c) on a plurality of known virus strains, wherein a match identifies the virus strain.

3. The method of claim 1 wherein the sequences to which the at least one pair of oligonucleotide primers hybridize are highly conserved.

4. The method of claim 1 wherein the amplifying step comprises polymerase chain reaction.

5. The method of claim 1 wherein the amplifying step comprises ligase chain reaction or strand displacement amplification.

6. The method of claim 1 wherein the virus strain is an arenavirus, bunyavirus, monoegaviras, picornavirus, astrovirus, calcivirus, nidovirus, flavivirus, togavirus, or retrovirus.

7. The method of claim 1 wherein the virus strain is a sabia virus, lassa vever virus, Machupo Virus, Argentine hemorrhagic fever virus, flexal virus, hantavirus, nairovirus, phlebovirus, Hantaan virus, Congo-crimean hemorrhagic fever virus, Rift valley fever virus, filovirus, paramyxovirus, ebola virus, Marburg virus, Equine morbillivirus, coxsackievirus, echovirus, human coxsackievirus A, human echovirus, human enterovirus, human poliovirus, hepatitis A virus, human parechovirus, human rhinovirus, human astrovirus, chiva virus, chitta virus, human calcivirus, norwalk virus, human coronavirus, human torovirus, Alfuy virus, Alkhurma virus, Apoi virus, Aroa virus, Bagaza virus, Banzi virus, Batu cave virus, Bouboui virus, Bukalasa bat virus, Bussliquara virus, Cacipacore virus, Carey island virus, Cowbone ridge virus, Dakar bat virus, Deer tick virus, Dengue virus type 1, Dengue virus type 2, Dengue virus type 3, Dengue virus type 4, Edge hill virus, Entebbe bat virus, Flavivirus sp., Gadgets gully virus, Hepatitis C virus, Iguape virus, Ilheus virus, Israel turkey meningoencephalitis virus, Japanese encephalities virus, Jugra virus, Jutiapa virus, Kadam virus, Kedougou virus, Kokobera virus, Koutango virus, Kunjin virus, Kyasanur forest disease virus, Langat aviurs, Louping III virus, Maeban virus, Modoc virus, Montana myotic leukoencephalitis virus, Murray Valley encephalitis virus, Naranjal virus, Negishi virus, Ntaya virus, Omsk hemorrhagic fever virus, Phnom-Penh bat virus, Potiskum virus, **Powassan virus**, Rio bravo virus, Rocio virus, Royal farm virus, Russian spring-summer encephalitis virus, Saboya virus, Saint Louis encephalitis virus, Sal vieja virus, San perlita virus, Saumarez reef virus, Sepik virus, Sitiawan virus, Sokuluk virus, Spondweni virus, Stratford virus, Tembusu virus, Tick-borne encephalitis virus, Tyulenly virus, Uganda 5 virus, Usutu virus, West Nile virus, Yellow fever virus, Chikugunya virus, Eastern equine encephalitis virus, Mayaro virus, O'nyong-nyong virus, Ross river virus, Venezuelan equine encephalitis

virus, Rubella virus, hepatities E virus, human immuodefeciency virus, or hepatitis B virus.

8. The method of claim 1 wherein the nucleic acid is ribosomal RNA.

9. The method of claim 1 wherein the nucleic acid encodes RNase P or an RNA-dependent RNA polymerase.

10. The method of claim 1 wherein the amplification product is ionized prior to molecular mass or base composition determination.

11. The method of claim 1 further comprising the step of isolating nucleic acid from the virus prior to contacting the nucleic acid with the at least one pair of oligonucleotide primers.

12. The method of claim 1 further comprising the step of performing steps a) through d) using a different oligonucleotide primer pair and comparing the results to molecular mass or base composition of one or more amplification products obtained by performing steps a) through c) on a different plurality of known viruses from those in step d).

13. The method of claim 1 wherein the one or more molecular masses or base compositions are contained in a database of molecular masses or base compositions.

14. The method of claim 1 wherein the amplification product is ionized by electrospray ionization, matrix-assisted laser desorption or fast atom bombardment.

15. The method of claim 1 wherein the molecular mass or base composition is determined by mass spectrometry.

16. The method of claim 15 wherein the mass spectrometry is Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF, or triple quadrupole.

17. The method of claim 1 further comprising performing step b) in the presence of an analog of adenine, thymidine, guanosine or cytidine having a different molecular weight than adenosine, thymidine, guanosine or cytidine.

18. The method of claim 1 wherein the oligonucleotide primer comprises a base analog at positions 1 and 2 of each triplet within the primer, wherein the base analog binds with increased affinity to its complement compared to the native base.

19. The method of claim 18 wherein the primer comprises a universal base at position 3 of each triplet within the primer.

20. The method of claim 18 wherein the base analog is a 2,6-diaminopurine, propyne T, propyne G, phenoxazine, or G-clamp.

21. The method of claim 19 wherein the universal base is an inosine, guanidine uridine, 5-nitroindole, 3-nitropyrrole, dP, dK, or 1-(2-deoxy-β-D-ribofuranosyl)-imidazole-4-carboxamide.

22. A method of distinguishing a first virus strain in a sample from at least a second virus strain comprising: a) contacting nucleic acid from the first virus strain in the sample with at least one pair of oligonucleotide primers which hybridize to sequences of the nucleic acid, wherein the sequences flank a variable acid sequence of the first virus strain; b) amplifying the variable nucleic acid sequence to produce an amplification product; c) determining the molecular mass or base composition of the amplification product; and d) distinguishing the first virus strain.

23. The method of claim 22 wherein step d) comprises comparing the molecular mass or base composition of the amplification product to the molecular mass or base composition of an amplification product from the at least second virus strain obtained by performing steps a) through c) on the at least second virus strain, wherein a different molecular mass for the amplification product of the first virus strain compared to the molecular mass of the at least second virus strain distinguishes the first virus strain.

24. The method of claim 22 wherein the virus strain is a hepatitis virus, a human rhinovirus, an encephalitis virus, or a human immuodefeciency virus.

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     (FILE 'HOME' ENTERED AT 15:35:18 ON 23 FEB 2004)

     FILE 'USPATFULL' ENTERED AT 15:35:57 ON 23 FEB 2004
L1           661 S YELLOW FEVER VIRUS
L2            59 S L1 AND (YELLOW FEVER VIRUS/CLM)
L3            17 S L2 AND (PRM AND E)
L4             4 S L3 AND (PRM/CLM)
L5            13 S L3 NOT L4
L6             7 S L3 AND E/CLM
L7           231 S ST. LOUIS ENCEPHALITIS VIRUS
L8            16 S L7 AND LOUIS/CLM
L9            13 S L8 NOT L5
L10           52 S POWASSAN VIRUS
L11            6 S L10 AND POWASSAN/CLM
L12            2 S L11 NOT L9

=> d l12,cbib,ab,clm,2

L12  ANSWER 2 OF 2  USPATFULL on STN
2003:30900 Nucleic acid vaccines for prevention of flavivirus infection.
    Chang, Gwong-Jen J., Fort Collins, CO, UNITED STATES
    US 2003022849 A1 20030130
    APPLICATION: US 2001-826115  A1  20010404 (9)
    PRIORITY: US 1998-87908P  19980604 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The present invention encompasses isolated nucleic acids containing
       transcriptional units which encode a signal sequence of one flavivirus
       and an immunogenic flavivirus antigen of a second flavivirus. The
       invention further encompasses a nucleic acid and protein vaccine and the
       use of the vaccine to immunize a subject against flavivirus infection.
       The invention also provides antigens encoded by nucleic acids of the
       invention, antibodies elicited in response to the antigens and use of
       the antigens and/or antibodies in detecting flavivirus or diagnosing
       flavivirus infection.
CLM    What is claimed is:
       1. An isolated nucleic acid comprising a transcriptional unit encoding a
       signal sequence of a structural protein of a first flavivirus and an
       immunogenic flavivirus antigen of a second flavivirus, wherein the
       transcriptional unit directs the synthesis of the antigen.

       2. The nucleic acid of claim 1, wherein the signal sequence is a
       Japanese encephalitis virus signal sequence.

       3. The nucleic acid of claim 1, wherein the immunogenic flavivirus
       antigen is of a flavivirus selected from the group consisting of yellow
       fever virus, dengue serotype 1 virus, dengue serotype 2 virus, dengue
       serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus,
       Powassan virus and West Nile virus.

       4. The nucleic acid of claim 1, wherein the transcriptional unit encodes
       a signal sequence of Japanese encephalitis virus and an M protein and an
       E protein of West Nile virus.

       5. The nucleic acid of claim 1, wherein the transcriptional unit encodes
       a signal sequence of Japanese encephalitis virus and an M protein and an
       E protein of yellow fever virus.

       6. The nucleic acid of claim 1, wherein the transcriptional unit encodes
       a signal sequence of Japanese encephalitis virus and an M protein and an
       E protein of St. Louis encephalitis virus.

       7. The nucleic acid of claim 1, wherein the transcriptional unit encodes
       a signal sequence of Japanese encephalitis virus and an M protein and an
       E protein of Powassan virus.

       8. The nucleic acid of claim 1, wherein the antigen is selected from the
       group consisting of an M protein of a flavivirus, an E protein of a
       flavivirus, both an M protein and an E protein of a flavivirus, a
       portion of an M protein of a flavivirus, a portion of an E protein of a
       flavivirus and both a portion of an M protein of a flavivirus and a
       portion of an E protein of a flavivirus or any combination thereof.

       9. The nucleic acid of claim 8, wherein the antigen is both the M
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protein and the E protein of a flavivirus.

10. The nucleic acid of claim 1, wherein the nucleic acid is DNA.

11. The nucleic acid of claim 10, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:19, SEQ ID NO:21 and SEQ ID NO:23.

12. The nucleic acid of claim 1, wherein the transcriptional unit comprises a control sequence disposed appropriately such that it operably controls the synthesis of the antigen.

13. The nucleic acid of claim 12, wherein the control sequence is the cytomegalovirus immediate early promoter.

14. The nucleic acid of claim 1, comprising a Kozak consensus sequence located at a translational start site for a polypeptide comprising the antigen encoded by the TU.

15. The nucleic acid of claim 1 wherein the transcriptional unit comprises a poly-A terminator.

16. A cell comprising the nucleic acid of claim 1.

17. A composition comprising the nucleic acid of claim 1 and a pharmaceutically acceptable carrier.

18. A method of immunizing a subject against infection by a flavivirus, comprising administering to the subject an effective amount of the composition of claim 17.

19. The method of claim 18, wherein the flavivirus antigen is of a flavivirus selected from the group consisting of yellow fever virus, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, **Powassan virus** and West Nile virus.

20. The method of claim 18, wherein the antigen is selected from the group consisting of an M protein of a flavivirus, an E protein of a flavivirus, both an M protein and an E protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an E protein of a flavivirus and both a portion of an M protein of a flavivirus and a portion of an E protein of a flavivirus or any combination thereof.

21. The method of claim 20, wherein the antigen is both the M protein and the E protein of a flavivirus, and wherein a cell within the body of the subject, after incorporating the nucleic acid within it, secretes subviral particles comprising the M protein and the E protein.

22. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of West Nile virus.

23. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of yellow fever virus.

24. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of St. Louis encephalitis virus.

25. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of **Powassan virus**.

26. The method of claim 18, comprising administering the composition to the subject in a single dose.

27. The method of claim 18, wherein the composition is administered via a parenteral route.

28. The nucleic acid of claim 1, wherein the antigen is a St. Louis encephalitis virus antigen.

29. The method of claim 18, wherein the antigen is a St. Louis encephalitis virus antigen.

30. The nucleic acid of claim 1, wherein the antigen is a Japanese encephalitis virus antigen.

31. The method of claim 18, wherein the antigen is a Japanese encephalitis virus antigen.

32. The nucleic acid of claim 1, wherein the antigen is a yellow fever virus antigen.

33. The method of claim 18, wherein the antigen is a yellow fever virus antigen.

34. The nucleic acid of claim 1, wherein the antigen is a dengue virus antigen.

35. The method of claim 18, wherein the antigen is a dengue virus antigen.

36. The nucleic acid of claim 1, wherein the antigen is a West Nile virus antigen.

37. The method of claim 18, wherein the antigen is a West Nile virus antigen.

38. An antigen produced from the nucleic acid of claim 1.

39. A method of detecting a flavivirus antibody in a sample, comprising: (a) contacting the sample with the antigen of claim 38 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby detecting a flavivirus antibody in the sample.

40. An antibody produced in response to immunization by the antigen of claim 38.

41. A method of detecting a flavivirus antigen in a sample, comprising: (a) contacting the sample with the antibody of claim 40 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby detecting a flavivirus antigen in a sample.

42. A method of diagnosing a flavivirus infection in a subject, comprising: (a) contacting a sample from the subject with the antigen of claim 38 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby diagnosing a flavivirus infection in a subject.

43. A method of diagnosing a flavivirus infection in a subject, comprising: (a) contacting a sample from the subject with the antibody of claim 40 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby diagnosing a flavivirus infection in a subject.

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     (FILE 'HOME' ENTERED AT 15:35:18 ON 23 FEB 2004)

     FILE 'USPATFULL' ENTERED AT 15:35:57 ON 23 FEB 2004
L1           661 S YELLOW FEVER VIRUS
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L3            17 S L2 AND (PRM AND E)
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L5            13 S L3 NOT L4
L6             7 S L3 AND E/CLM
L7           231 S ST. LOUIS ENCEPHALITIS VIRUS
L8            16 S L7 AND LOUIS/CLM
L9            13 S L8 NOT L5
L10           52 S POWASSAN VIRUS
L11            6 S L10 AND POWASSAN/CLM
L12            2 S L11 NOT L9

=> s west nile virus
         42398 WEST
          1892 NILE
         70100 VIRUS
L13        264 WEST NILE VIRUS
               (WEST(W)NILE(W)VIRUS)

=> s l13 and (west nile virus/clm)
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          674 WEST/CLM
          163 NILE/CLM
        11930 VIRUS/CLM
           34 WEST NILE VIRUS/CLM
                ((WEST(W)NILE(W)VIRUS)/CLM)
L14        34 L13 AND (WEST NILE VIRUS/CLM)

=> d l14,cbib,ab,clm,20-34

L14  ANSWER 20 OF 34  USPATFULL on STN
2003:180684 Simultaneous quantification of nucleic acids in diseased cells.
    Stuyver, Lieven, Snellville, GA, UNITED STATES
    US 2003124512 A1 20030703
    APPLICATION: US 2001-8140  A1  20011018 (10)
    PRIORITY: US 2000-241488P  20001018 (60)
    US 2000-256067P  20001215 (60)
    US 2001-282156P  20010406 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     This invention pertains to a novel method to screen the efficacy of
       various anti-viral, and especially anti-HIV and HCV agents by using a
       novel real-time polymerase chain reaction technique. This method can
       also be applied to toxicity screening, especially of mitochondrial
       toxicity of these compounds as well.
CLM    What is claimed is:
       1. A process for identifying a compound which inhibits viral replication
       that includes contacting nucleic acids from a virus infected host with
       an amplification reaction mixture that contains at least two primers
       and/or probes that provide detectable signals during a polymerase chain
       reaction, wherein  the first primer and/or probe provides a detectable
       signal on the occurrence of the transcription of viral nucleic acids;
       and  the second primer and/or probe provides a second detectable signal
       on the occurrence of the transcription of host nucleic acids.

       2. The process of claim 1, wherein the host nucleic acid is nuclear
       nucleic acid.

       3. The process of claim 1, wherein the host nucleic acid is
       mitochondrial nucleic acid.

       4. The process of claim 3, wherein the mitochondrial nucleic acid is
       mitochondrial DNA.

       5. The process of claim 3, wherein the mitochondrial nucleic acid is
       mitochondrial RNA.

       6. The process of claim 1, wherein the viral nucleic acid is a
       non-coding sequence.

       7. The process of claim 6, wherein the non-coding sequence is a
       5'-non-coding sequence.

       8. The process of claim 6, wherein the non-coding sequence is a
       3'-non-coding sequence.

       9. The process of claim 6, wherein the non-coding sequence is an intron.

       10. The process of claim 6, wherein the non-coding sequence is from
       β-actin.

       11. The process of claim 6, wherein the non-coding sequence is from
       GAPDH.

       12. The process of claim 1, wherein the viral nucleic acid is a coding
       sequence.

       13. The process of claim 12, wherein the coding sequence is from HIV.

       14. The process of claim 12, wherein the coding sequence is from HBV.

       15. The process of claim 12, wherein the coding sequence is from HCV.

       16. The process of claim 12, wherein the coding sequence is from BVDV.

       17. The process of claim 12, wherein the coding sequence is from West
       Nile Virus.

       18. The process of claim 12, wherein the coding sequence is from herpes.
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19. The process of claim 12, wherein the coding sequence is from influenza.

20. The process of claim 12, wherein the coding sequence is from RSV.

21. The process of claim 12, wherein the coding sequence is from EBV.

22. The process of claim 12, wherein the coding sequence is from CMV.

23. A process for assessing the toxicity of a compound that includes contacting nucleic acids from a host with an amplification reaction mixture that contains at least two primers and/or probes that provide detectable signals during a polymerase chain reaction, wherein the first primer and/or probe provides a detectable signal on the occurrence on the transcription of host mitochondrial nucleic acids; and the second primer and/or probe provides a second detectable signal on the occurrence on the transcription of host nuclear nucleic acid.

24. The process of claim 23, wherein the host mitochondrial nucleic acid is mitochondrial DNA.

25. The process of claim 23, wherein the host mitochondrial nucleic acid is mitochondrial RNA.

26. The process of claim 23, wherein the host mitochondrial nucleic acid is a non-coding sequence.

27. The process of claim 26, wherein the non-coding sequence is a 5'-non-coding sequence.

28. The process of claim 26, wherein the non-coding sequence is a 3'-non-coding sequence.

29. The process of claim 26, wherein the non-coding sequence is an intron.

30. The process of claim 26, wherein the non-coding sequence is from β-actin.

31. The process of claim 26, wherein the non-coding sequence is from GAPDH.

32. The process of claim 23, wherein the host mitochondrial nucleic acid is a coding sequence.

L14 ANSWER 21 OF 34 USPATFULL on STN
2003:159247 Method for large scale production of virus antigen.
Reiter, Manfred, Vienna, AUSTRIA
Mundt, Wolfgang, Vienna, AUSTRIA
US 2003108860 A1 20030612
APPLICATION: US 2001-6881 A1 20011210 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides improved methods of production of viral antigen on a culture of adherent cells bound to a microcarrier, wherein the methods provide for increased viral antigen yield per culture medium volume. The invention is also directed to a cell culture biomass of adherent cells having increased cell density and microcarrier concentration compared to the respective confluent cell culture.

CLM What is claimed is:

1. A method for production of virus or viral antigen, comprising the steps of (a) providing a culture of adherent cells bound to a microcarrier, (b) growing the cell culture to confluence, (c) infecting the cells with a virus and (d) incubating said culture of cells infected with said virus to propagate said virus, wherein the cell density of the biomass of the cell culture grown to confluence is increased (i) prior to step (c) or (ii) after step (c) and maintained at high cell density during step (d).

2. The method according to claim 1, wherein the density of the cell culture grown to confluence is concentrated at least about 1.3 fold.

3. The method according to claim 1, wherein the cell density of the cell culture grown to confluence is between about 0.6×10^6 and about 7.0×10^6 cells/ml.

4. The method according to claim 1, wherein the microcarrier is selected from the group of microcarriers made of dextran, collagen, polystyrene,

polyacrylamide, gelatine, glass, cellulose, polyethylene and plastic.

5. The method according to claim 1, wherein the microcarrier concentration in the culture of cells of step (a) is between about 0.5 g/l and about 14 g/l.

6. The method according to claim 1, wherein said cells are selected from the group of adherent cells of VERO, BHK, CHO, RK, RK44, RK13, MRC-5, MDCK, CEF or diploid monolayer cells.

7. The method according to claim 1, wherein said cells bound to a microcarrier are grown in serum free medium.

8. The method according to claim 1, wherein said cells bound to a microcarrier are grown in serum and protein free medium.

9. The method according to claim 1, wherein the virus is selected from the group of Influenza virus, Ross River Virus, Hepatitis A Virus, Vaccinia Virus and recombinant derivatives thereof, Herpes Simplex Virus, Japanese encephalitis Virus, **West Nile Virus**, Yellow Fever Virus and chimeric thereof, Rhinovirus and Reovirus.

10. The method according to claim 1, further comprising the step (e) harvesting the virus propagated.

11. A method for production of purified virus or virus antigen comprising the steps of (a) providing a culture of adherent cells bound to a microcarrier, (b) growing the cell culture to confluence, (c) infecting the culture of cells with a virus, (d) incubating said culture of cells infected with said virus to propagate said virus (e) harvesting the virus produced and (f) purifying said virus harvested, wherein the cell density of the biomass of the cell culture grown to confluence is increased (i) prior to step (c) or (ii) after step (c) and maintained at high cell density during step (d).

12. The method according to claim 11, wherein the virus produced is harvested from the cell culture supernatant.

13. The method according to claim 11, wherein the virus produced is harvested from the cell biomass.

14. A method for production of Influenza virus, comprising the steps of (a) providing a culture of adherent cells bound to a microcarrier, (b) growing the cell culture to confluence, (c) infecting the cells with an Influenza virus and (d) incubating said culture of cells infected with said Influenza virus to propagate said virus, wherein the cell density of the biomass of the cell culture grown to confluence is increased (i) prior to step (c) or (ii) after step (c) and maintained at high cell density during step (d).

15. The method according to claim 14, wherein said cells are VERO cells.

16. The method according to claim 14, wherein said cells are MDCK cells.

17. The method according to claim 14, wherein said cells bound to a microcarrier are grown in serum free medium.

18. The method according to claim 14, wherein said cells bound to a microcarrier are grown in serum and protein free medium.

19. The method according to claim 14, wherein the cell culture grown to confluence is concentrated at least about 1.3 fold.

20. The method according to claim 14, wherein further comprising the step (e) of harvesting said Influenza virus or Influenza virus antigen produced.

21. The method according to claim 14, further comprising the step (f) of purifying said Influenza virus harvested.

22. A cell culture biomass of adherent cells bound to a microcarrier, wherein the biomass of cells in said cell culture is at least about 1.3 fold compared to a cell culture that has been grown to confluence.

23. The culture according to claim 22, wherein said cells are VERO cells.

24. The culture according to claim 22, wherein said culture is serum free.

25. The culture according to claim 22, wherein said culture is serum and protein free.

26. The culture according to claim 22, wherein said cells are infected with a virus.

27. A cell culture biomass of VERO cells bound to a microcarrier, wherein the biomass of the VERO cells in said cell culture is at least about 1.3-fold compared to a VERO cell culture that has been grown to confluence.

28. The culture according to claim 27, wherein said culture is serum free.

29. The culture according to claim 27, wherein said culture is serum and protein free.

30. A cell culture biomass of adherent cells bound to a microcarrier infected with a virus, wherein the biomass of the infected cells in said cell culture is at least about 1.3-fold compared to a cell culture that has been grown to confluence prior to infection.

31. The culture according to claim 30, wherein said culture is serum free.

32. The culture according to claim 30, wherein said culture is serum and protein free.

33. The culture according to claim 30, wherein said cells are VERO cells.

34. A cell culture biomass of VERO cells bound to a microcarrier and cells infected with a virus, wherein the biomass of the VERO cells in said cell culture is at least about 1.3-fold compared to a VERO cell culture that has been grown to confluence.

35. A cell culture according to claim 34, infected with a virus selected from the group of Influenza virus, Ross River Virus, Hepatitis A Virus, Vaccinia Virus and recombinant derivatives thereof, Herpes Simplex Virus, Japanese encephalitis Virus, **West Nile Virus**, Yellow Fever Virus and chimeric thereof, Rhinovirus and Reovirus.

36. A cell culture biomass of VERO cells bound to a microcarrier and infected with a Influenza virus wherein the biomass of the VERO cells in said cell culture is at least about 1.3-fold compared to a VERO cell culture that has been grown to confluence.

37. A cell culture biomass of VERO cells bound to a microcarrier and infected with a Ross River Virus, wherein the biomass of the VERO cells in said cell culture is at least about 1.3-fold compared to a VERO cell culture that has been grown to confluence.

38. A cell culture biomass of VERO cells bound to a microcarrier and infected with a Vaccinia Virus, wherein the biomass of the VERO cells in said cell culture is at least about 1.3-fold compared to a VERO cell culture that has been grown to confluence.

L14 ANSWER 22 OF 34 USPATFULL on STN
2003:155723 Polynucleotides encoding flavivirus and alphavirus multivalent antigenic polypeptides.
Punnonen, Juha, Palo Alto, CA, United States
Bass, Steven H., Hillsborough, CA, United States
Whalen, Robert Gerald, Paris, FRANCE
Howard, Russell, Los Altos Hills, CA, United States
Stemmer, Willem P. C., Los Gatos, CA, United States
Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)
US 6576757 B1 20030610
APPLICATION: US 2000-724852 20001128 (9)
PRIORITY: US 1998-105509P 19981023 (60)
US 1998-74294P 19980211 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention is directed to antigen library immunization, which provides methods for obtaining antigens having improved properties for therapeutic and other uses. The methods are useful for obtaining improved antigens that can induce an immune response against pathogens, cancer, and other conditions, as well as antigens that are effective in

modulating allergy, inflammatory and autoimmune diseases.

CLM What is claimed is:

1. A nucleic acid comprising a polynucleotide sequence encoding a recombinant multivalent antigenic polypeptide that comprises multiple non-contiguous subsequences of at least a first antigenic polypeptide of at least a first flavivirus or alphavirus and multiple non-contiguous subsequences of at least a second antigenic polypeptide of at least a second flavivirus or alphavirus, each subsequence being positioned relative to its position in the respective antigenic polypeptide, wherein the recombinant multivalent antigenic polypeptide induces an immune response against the first and second antigenic polypeptides that is greater than the immune response induced by any one of the fist and second antigenic polypeptides against any other of the first and second antigenic polypeptides.

2. The nucleic acid of claim 1, wherein the multivalent antigenic polypeptide comprises multiple non-contiguous subsequences of at least a third antigenic polypeptide of at least a third flavivirus or alphavirus.

3. The nucleic acid of claim 1, wherein at least the first and second antigenic polypeptides are from a virus selected from the group consisting of a Venezuelan equine encephalitis virus or a related alphavirus, a virus of the Japanese encephalitis virus complex, a virus of the tick-borne encephalitis virus complex, a Dengue virus, a yellow fever virus, a St. Louis encephalitis virus, and a Murray Valley encephalitis virus, Kunjin virus, and **West Nile virus**.

4. The nucleic acid of claim 1, wherein each of at least the first and second antigenic polypeptides comprises an envelope protein, a premembrane protein, or both an envelope protein and a premembrane protein.

5. The nucleic acid of claim 1, wherein at least the first and second antigenic polypeptides are different serotypes of a flavivirus or alphavirus.

6. The nucleic acid of claim 1, wherein at least the first and second antigenic polypeptides are different species or strains of a flavivirus or alphavirus.

7. A vector comprising the nucleic acid of claim 1.

8. The vector of claim 7, wherein the vector comprises an expression vector.

9. A host cell comprising the nucleic acid of claim 1.

10. A host cell comprising the vector of claim 7.

11. The host cell of claim 9, wherein the host cell is in vivo.

12. The host cell of claim 9, wherein the host cell expresses a polypeptide encoded by the nucleic acid.

13. A method of producing a recombinant multivalent antigenic polypeptide comprising culturing a host cell comprising the expression vector of claim 8 under conditions suitable for expression of the multivalent antigenic polypeptide.

14. The method of claim 13, further comprising isolating the multivalent antigenic polypeptide.

15. A composition comprising the nucleic acid of claim 1 and an excipient.

16. The nucleic acid of claim 1, wherein the multivalent antigenic polypeptide induces an immune response to the first and second antigenic polypeptides that is greater than the immune response induced by any one of the first and second antigenic polypeptides against any of the first and second antigenic polypeptides.

17. The nucleic acid of claim 1, wherein the multivalent antigenic polypeptide induces an immune response that is cross reactive against at least the first and second antigenic polypeptides and at least a third antigenic polypeptide of a flavivirus or alphavirus.

18. The nucleic acid of claim 1, wherein the multivalent antigenic polypeptide induces an immune response that is cross reactive against at

least two different serotypes, strains, or species of a flavivirus or alphavirus.

19. The nucleic acid of claim 18, wherein the multivalent antigenic polypeptide induces an immune response that is cross reactive against at least three different serotypes, strains, or species of a flavivirus or alphavirus.

20. The nucleic acid of claim 19, wherein the multivalent antigenic polypeptide induces an immune response against a disease condition caused by one or more of at least three different serotypes, strains, or species.

21. A nucleic acid which encodes a recombinant multivalent antigenic polypeptide comprising multiple non-contiguous subsequences of at least a first antigenic polypeptide of at least a first flavivirus or alphavirus and multiple non-contiguous subsequences of at least a second antigenic polypeptide of at least a second flavivirus or alphavirus, each subsequence being positioned relative to its position in the respective antigenic polypeptide, wherein the recombinant multivalent antigenic polypeptide is prepared by a method comprising: (1) recombining at least a first nucleic acid comprising a nucleotide sequence that encodes the first antigenic polypeptide and at least a second nucleic acid comprising a nucleotide sequence that encodes the second antigenic polypeptide, wherein at least the first and second nucleic acids differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids; and (2) screening the library of recombinant nucleic acids for at least one recombinant nucleic acid that encodes a recombinant multivalent antigenic polypeptide that induces an immune response against each of the first and second flaviviruses or alphaviruses that is greater than the immune response induced by the first antigenic polypeptide against the second flavivirus or alphavirus and the immune response induced by the second antigenic polypeptide against the first flavivirus or alphavirus.

22. The nucleic acid of claim 21, wherein the method further comprises: (3) recombining at least one recombinant nucleic acid with at least a third nucleic acid comprising a nucleotide sequence that encodes a third antigenic polypeptide of a third flavivirus or alphavirus, wherein the third nucleic acid is the same or different from at least the first and second nucleic acids, to produce a further library of recombinant nucleic acids; (4) screening the further library of recombinant nucleic acids for at least one further recombinant nucleic acid that encodes a recombinant multivalent antigenic polypeptide that induces an immune response against each of the first, second and third flaviviruses or alphaviruses that is greater than the immune response induced by (i) the first antigenic polypeptide against the second or third flavivirus or alphavirus, (ii) the second antigenic polypeptide against the first or third flavivirus or alphavirus, and (iii) the third antigenic polypeptide against the first or second flavivirus or alphavirus; and (5) repeating (3) and (4), as necessary, for a further recombinant multivalent antigenic polypeptide that induces an immune response against each of the first, second and third flaviviruses or alphaviruses that is greater than the immune response induced by (i) the first antigenic polypeptide against the second or third flavivirus or alphavirus, (ii) the second antigenic polypeptide against the first or third flavivirus or alphavirus, and (iii) the third antigenic polypeptide against the first or second flavivirus or alphavirus.

23. The nucleic acid of claim 21, wherein at least the first and second antigenic polypeptides are different serotypes, species or strains of a flavivirus or alphavirus.

24. The nucleic acid of claim 22, wherein at least first, second, and third antigenic polypeptides are different serotypes, species or strains of a flavivirus or alphavirus.

25. The nucleic acid of claim 1, wherein the multivalent antigenic polypeptide induces an immune response against the first and second flaviviruses or alphaviruses that is greater than the immune response induced by the first antigenic polypeptide against the second flavivirus or alphavirus and the immune response induced by the second antigenic polypeptide against the first flavivirus or alphavirus.

26. The nucleic acid of claims 25, wherein the multivalent antigenic polypeptide induces an immune response against the fist and second flaviviruses or alphaviruses that is greater than the immune response induced by each of the first and second antigenic polypeptides against the fist flavivirus or alphavirus and the second flavivirus or

alphavirus.

27. The nucleic acid of claim 1, wherein the multivalent antigenic polypeptide induces production of neutralizing antibodies against at least each of the first and second antigenic polypeptides.

28. The nucleic acid of claim 1, wherein the multivalent antigenic polypeptide induces production of neutralizing antibodies against at least the first flavivirus or alphavirus and the second flavivirus or alphavirus.

29. The nucleic acid of claim 2, wherein the multivalent antigenic polypeptide induces production of neutralizing antibodies against at least each of the first, second, and third antigenic polypeptides.

30. The nucleic acid of claim 2, wherein the multivalent antigenic polypeptide induces production of neutralizing antibodies against at least the first, second, and third flaviviruses or alphaviruses.

31. The nucleic acid of claim 2, wherein the multivalent antigenic polypeptide induces an immune response against at least the first, second, and third flaviviruses or alphaviruses.

32. The nucleic acid of claim 2, wherein the multivalent antigenic polypeptide induces an immune response against the first, second, and third antigenic polypeptides that is greater than the immune response induced by any one of the first, second, and third antigenic polypeptides against any other of the first, second, and third antigenic polypeptides.

33. A nucleic acid that encodes a multivalent antigenic polypeptide comprising multiple non-contiguous subsequences of at least a first antigenic polypeptide of at least a first flavivirus or alphavirus and multiple non-contiguous subsequences of at least a second antigenic polypeptide of at least a second flavivirus or alphavirus, each subsequence being positioned relative to its position in the respective antigenic polypeptide, wherein the multivalent antigenic polypeptide induces production of neutralizing antibodies against at least the first and second antigenic polypeptides.

34. The nucleic acid of claim 33, wherein the multivalent antigenic polypeptide induces production of neutralizing antibodies against at least the first and second flaviviruses or alphaviruses.

35. The nucleic acid of claim 33, wherein each of the first and second antigenic polypeptides comprises an envelope protein, a premembrane protein, or both an envelope protein and a premembrane protein.

36. The nucleic acid of claim 33, wherein the multivalent antigenic polypeptide is present as a component of a virus or a viral vector.

37. A composition comprising the nucleic acid of claim 33, and a carrier.

38. A vector comprising the nucleic acid of claim 33.

39. A host cell comprising the nucleic acid of claim 33.

40. A nucleic acid that encodes a multivalent antigenic polypeptide comprising multiple non-contiguous subsequences of a fast antigenic polypeptide of a dengue-1 virus, multiple non-contiguous subsequences of a second antigenic polypeptide of a dengue-2 virus, multiple non-contiguous subsequences of a third antigenic polypeptide of a dengue-3 virus, and multiple noncontiguous subsequences of a fourth antigenic polypeptide of a dengue-4 virus, each subsequence being positioned relative to its position in the first, second, third, or fourth antigenic polypeptide, wherein the multivalent antigenic polypeptide induces production of neutralizing antibodies against the dengue-1 virus, dengue-2 virus, dengue-3 virus, and dengue-4 virus.

41. The nucleic acid of claim 40, wherein each of the first, second, third, and four antigenic polypeptides comprises an envelope protein, a premembrane protein, or both an envelope protein and a premembrane protein.

42. The nucleic acid of claim 40, wherein the multivalent antigenic polypeptide is present as a component of a virus or a viral vector.

43. A composition comprising the nucleic acid of claim 40 and a carrier.

44. A vector composing the nucleic acid of claim 40.

45. A host cell comprising the nucleic acid of claim 40.

46. The host cell of claim 10, wherein the host cell is in vivo.

47. The host cell of claim 10, wherein the host cell expresses a polypeptide encoded by the nucleic acid.

48. The host cell of claim 11, wherein the host cell expresses a polypeptide encoded by the nucleic acid.

49. The nucleic acid of claim 1, wherein the multivalent antigenic polypeptide induces an immune response against each of the at least first and second antigenic polypeptides that is greater than the immune response induced by any one of the at least first and second antigenic polypeptides against any other of the at least first and second antigenic polypeptides.

50. The nucleic acid of claim 33, wherein the multivalent antigenic polypeptide further comprises multiple non-contiguous subsequences of at least a third antigenic polypeptide of at least a third flavivirus or alphavirus.

51. The nucleic acid of claim 50, wherein the multivalent antigenic polypeptide induces production of neutralizing antibodies against at least the first, second, and third antigenic polypeptides.

52. The nucleic acid of claim 50, wherein the multivalent antigenic polypeptide induces production of neutralizing antibodies against at least the first, second, and third flaviviruses or alphaviruses.

53. The nucleic acid of claim 50, wherein the multivalent antigenic polypeptide induces production of neutralizing antibodies against each of the at least first, second, and third antigenic polypeptides or each of the at least first, second, and third flaviviruses or alphaviruses.

54. The nucleic acid of claim 2, wherein the multivalent antigenic polypeptide induces an immune response against the first, second, and third flaviviruses or alphaviruses that is greater than the immune response induced by any one of he first, second, and third antigenic polypeptides against any other of the first, second, and flaviviruses or alphaviruses.

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2003:153317 Conjugates and compositions for cellular delivery.
Matulic-Adamic, Jasenka, Boulder, CO, UNITED STATES
Beigelman, Leonid, Longmont, CO, UNITED STATES
US 2003104985 A1 20030605
APPLICATION: US 2002-151116 A1 20020517 (10)
PRIORITY: US 2002-362016P 20020306 (60)
US 2001-292217P 20010518 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention features conjugates, compositions, methods of synthesis, and applications thereof, including folate derived conjugates of nucleosides, nucleotides, non-nucleosides, and nucleic acids including enzymatic nucleic acids and antisense nucleic acid molecules.
CLM What is claimed is:
1. A compound having the formula I: ##STR52## wherein each R_1, R_3, R_4, R_5, R_6, R_7 and R_8 is independently hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker.

2. A compound having the formula II: ##STR53## wherein each R_3, R_4, R_5, R_6 and R_7 is independently hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker.

3. A compound having the formula III: ##STR54## wherein each R_1, R_3, R_4, R_5, R_6 and R_7 is independently hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, or nucleic acid.

4. A compound having the formula IV: ##STR55## wherein each R_3, R_4, R_5, R_6 and R_7 is independently hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker, and R_{13} is an amino acid side chain.

5. A compound having the formula V: ##STR56## wherein each R1 and R4 is independently a protecting group or hydrogen, each R_3, R_5, R_6, R_7 and R_8 is independently hydrogen, alkyl or nitrogen protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each R_9 and R_{10} is independently a nitrogen containing group, cyanoalkoxy, alkoxy, aryloxy, or alkyl group.

6. A compound having the formula VI: ##STR57## wherein each R_4, R_5, R_6 and R_7 is independently hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, or a protecting group, R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker, each "n" is independently an integer from 0 to about 200, and L is a degradable linker.

7. A compound having the formula VII: ##STR58## wherein each R_1, R_3, R_4, R_5, R_6 and R_7 is independently hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, or a protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and R_2 is a phosphorus containing group, nucleoside, nucleotide, small molecule, nucleic acid, or a solid support comprising a linker.

8. A compound having the formula VIII: ##STR59## wherein each R1 and R4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each R_9 and R_{10} is independently a nitrogen containing group, cyanoalkoxy, alkoxy, aryloxy, or alkyl group.

9. A method for synthesizing a compound having Formula V: ##STR60## wherein each R1 and R4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, each "n" is independently an integer from 0 to about 200, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each R_9 and R_{10} is independently a nitrogen containing group, cyanoalkoxy, alkoxy, aryloxy, or alkyl group, comprising: (a) coupling a bis-hydroxy aminoalkyl derivative with a N-protected aminoalkanoic acid to yield a compound of Formula IX; ##STR61## wherein R_{11} is an amino protecting group, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200; (b) introducing primary hydroxy protection followed by amino deprotection to yield a deprotected amine of Formula X: ##STR62## wherein R_1 is a protecting group, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200; (c) coupling the deprotected amine with a protected amino acid to yield a compound of Formula XI: ##STR63## wherein each R1 and R4 is independently a protecting group or hydrogen, each "n" is independently an integer from 0 to about 200, R_{11} is an amino protecting group, and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl; (d) deprotecting the amine of the compound of Formula XI to yield a compound of Formula XII: ##STR64## wherein each R1 and R4 is independently a protecting group or

hydrogen, each "n" is independently an integer from 0 to about 200, R11 is an amino protecting group, and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl; (e) coupling the compound of Formula XII with an amino protected pteroic acid to yield a compound of Formula XIII: ##STR65## wherein each R_1 and R_4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200; and (f) introducing a phosphorus containing group at the secondary hydroxyl to yield a compound of Formula V.

10. A method for synthesizing a compound having Formula VIII: ##STR66## wherein each R_1 and R_4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, each "n" is independently an integer from 0 to about 200, each R_9 and R_{10} is independently a nitrogen containing group, cyanoalkoxy, alkoxy, aryloxy, or alkyl group, and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, comprising; (a) coupling a bis-hydroxy aminoalkyl derivative with a protected amino acid to yield a compound of Formula XIV: ##STR67## wherein R_{11} is an amino protecting group, each "n" is independently an integer from 0 to about 200, R_4 is independently a protecting group, and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl; (b) introducing primary hydroxy protection followed by amino deprotection to yield a deprotected amine of Formula XV: ##STR68## wherein each R_1 and R_4 is independently a protecting group or hydrogen, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200; (c) coupling the deprotected amine with an amino protected pteroic acid to yield a compound of Formula XVI: ##STR69## wherein each R_1 and R_4 is independently a protecting group or hydrogen, each R_3, R_5, R_6 and R_7 is independently hydrogen, alkyl or nitrogen protecting group, R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl, and each "n" is independently an integer from 0 to about 200; and (f) introducing a phosphorus containing group at the secondary hydroxyl to yield a compound of Formula VIII.

11. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein R_2 is a phosphorus containing group.

12. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein R_2 is a nucleoside.

13. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein R_2 is a nucleotide.

14. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein R_2 is a small molecule.

15. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein R_2 is a nucleic acid.

16. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein R_2 is a solid support comprising a linker.

17. The compound of claim 12, wherein said nucleoside is a nucleoside with anticancer activity.

18. The compound of claim 12, wherein said nucleoside is a nucleoside with antiviral activity.

19. The compound of claim 12, wherein said nucleoside is fludarabine.

20. The compound of claim 12, wherein said nucleoside is lamivudine (3TC).

21. The compound of claim 12, wherein said nucleoside is 5-fluro uridine.

22. The compound of claim 12, wherein said nucleoside is AZT.

23. The compound of claim 12, wherein said nucleoside is ara-adenosine or ara-adenosine monophosphate.

24. The compound of claim 12, wherein said nucleoside is a dideoxy nucleoside analog.

25. The compound of claim 12, wherein said nucleoside is carbodeoxyguanosine.

26. The compound of claim 12, wherein said nucleoside is ribavirin.

27. The compound of claim 12, wherein said nucleoside is fialuridine.

28. The compound of claim 12, wherein said nucleoside is lobucavir.

29. The compound of claim 12, wherein said nucleoside is a pyrophosphate nucleoside analog.

30. The compound of claim 12, wherein said nucleoside is an acyclic nucleoside analog.

31. The compound of claim 12, wherein said nucleoside is acyclovir.

32. The compound of claim 12, wherein said nucleoside is gangciclovir.

33. The compound of claim 12, wherein said nucleoside is penciclovir.

34. The compound of claim 12, wherein said nucleoside is famciclovir.

35. The compound of claim 12, wherein said nucleoside is an L-nucleoside analog.

36. The compound of claim 12, wherein said nucleoside is FTC.

37. The compound of claim 12, wherein said nucleoside is L-FMAU.

38. The compound of claim 12, wherein said nucleoside is L-ddC or L-FddC.

39. The compound of claim 12, wherein said nucleoside is L-d4C or L-Fd4C.

40. The compound of claim 12, wherein said nucleoside is an L-dideoxypurine nucleoside analog.

41. The compound of claim 12, wherein said nucleoside is cytallene.

42. The compound of claim 12, wherein said nucleoside is bis-POM PMEA (GS-840).

43. The compound of claim 12, wherein said nucleoside is BMS-200,475.

44. The compound of claim 4, wherein R_{13} comprises an alkylamine.

45. The compound of claim 4, wherein R_{13} comprises an alkanol.

46. The compound of claim 4, wherein R_{13} comprises $--CH_{2O}--$.

47. The compound of claim 4, wherein R_{13} comprises $--CH(CH_2)CH_{2O}--$.

48. The compound of claim 6, wherein L is serine.

49. The compound of claim 6, wherein L is threonine.

50. The compound of claim 6, wherein L is a photolabile linkage.

51. The compound of any of claims 5 or 8, wherein R_9 comprises a phosphorus protecting group

52. The compound of claim 51, wherein said phosphorus protecting group is $--OCH_{2CH2CN}$ (oxyethylcyano).

53. The compound of any of claims 5 or 8, wherein R_{10} comprises a nitrogen containing group.

54. The compound of claim 53, wherein said nitrogen containing group is $--N(R_{14})$ wherein R_{14} is a straight or branched chain alkyl having form about 1 to 10 carbons.

55. The compound of any of claims 5 or 8, wherein R_{10} comprises a heterocycloalkyl or heterocycloalkenyl ring containing from about 4 to 7 atoms, and having up to 3 heteroatoms selected from oxygen, nitrogen, and sulfur.

56. The compound of any of claims 1, 5 or 8, wherein R_1 is an acid labile protecting group.

57. The compound of any of claims 1, 5 or 8, wherein R_1 is a trityl or substituted trityl group.

58. The compound of claim 57, wherein said substituted trityl group is a dimethoxytrityl or mono-methoxytrityl group.

59. The compound of any of claims 1, 2, 3, 4, 5, 6, 7 or 8, wherein R_4 is tert-butyl, Fm (fluorenyl-methoxy), or allyl.

60. The compound of any of claims 1, 2, 3, 4, 5, 6, 7 or 8, wherein R_6 is TFA (trifluoracetyl).

61. The compound of any of claims 1, 2, 3, 4, 5, 6, 7 or 8, wherein R_3, R_5, R_7 and R_8 are hydrogen.

62. The compound of any of claims 1, 2, 3, 4, 5, 6, 7 or 8, wherein R_7 is isobutyryl.

63. The compound of any of claims 1, 2, 3, 4, 5, 6, 7 or 8, wherein R_7 is dimethylformamide.

64. The compound of any of claims 1, 2, 3, 4, 5, 6, 7 or 8, wherein R_7 is hydrogen.

65. The compound of any of claims 1, 2, 3, 5, 7 or 8, wherein R_{12} is methyl.

66. The compound of any of claims 1, 2, 3, 5, 7 or 8, wherein R_{12} is ethyl.

67. The compound of any of claim 15, wherein said nucleic acid is an enzymatic nucleic acid.

68. The compound of claim 67, wherein said enzymatic nucleic acid is a hammerhead.

69. The compound of claim 67, wherein said enzymatic nucleic acid is an Inozyme.

70. The compound of claim 67, wherein said enzymatic nucleic acid is a DNAzyme.

71. The compound of claim 67, wherein said enzymatic nucleic acid is a G-cleaver.

72. The compound of claim 67, wherein said enzymatic nucleic acid is a Zinzyme.

73. The compound of claim 67, wherein said enzymatic nucleic acid is an Amberzyme.

74. The compound of claim 67, wherein said enzymatic nucleic acid is an allozyme.

75. The compound of any of claim 15, wherein said nucleic acid is an antisense nucleic acid.

76. The compound of any of claim 15, wherein said nucleic acid is a 2-5A nucleic acid chimera.

77. The compound of any of claim 15, wherein said nucleic acid is a decoy nucleic acid.

78. The compound of claim 13, wherein said nucleotide is a nucleotide with anticancer activity.

79. The compound of claim 13, wherein said nucleotide is a nucleotide with antiviral activity.

80. The compound of claim 16, wherein said solid support comprising a

linker of Formula XVII: ##STR70## wherein SS is a solid support, and each "n" is independently an integer from 1 to 200.

81. The compound of claim 80, wherein said solid support is controlled pore glass (CPG).

82. The compound of claim 80, wherein said solid support is polystyrene.

83. The compound of claim 16, wherein said compound is used in the synthesis of a nucleic acid.

84. A pharmaceutical composition comprising the compound of claim 1 in a pharmaceutically acceptable carrier.

85. A pharmaceutical composition comprising the compound of claim 2 in a pharmaceutically acceptable carrier.

86. A pharmaceutical composition comprising the compound of claim 3 in a pharmaceutically acceptable carrier.

87. A pharmaceutical composition comprising the compound of claim 4 in a pharmaceutically acceptable carrier.

88. A pharmaceutical composition comprising the compound of claim 6 in a pharmaceutically acceptable carrier.

89. A pharmaceutical composition comprising the compound of claim 7 in a pharmaceutically acceptable carrier.

90. A method of treating cancer in a patient, comprising contacting cells of said patient with the pharmaceutical composition of any of claims 84-89, under conditions suitable for said treatment.

91. The method of claim 90, further comprising the use of one or more other drug therapies under conditions suitable for said treatment.

92. The method of claim 90, wherein said cancer is breast cancer, lung cancer, colorectal cancer, brain cancer, esophageal cancer, stomach cancer, bladder cancer, pancreatic cancer, cervical cancer, head and neck cancer, ovarian cancer, melanoma, lymphoma, glioma, or multidrug resistant cancers.

93. A method of treating a patient infected with a virus, comprising contacting cells of said patient with the pharmaceutical composition of any of claims 84-89, under conditions suitable for said treatment.

94. The method of claim 93, further comprising the use of one or more other drug therapies under conditions suitable for said treatment.

95. The method of claim 93, wherein said virus is HIV, HBV, HCV, CMV, RSV, HSV, poliovirus, influenza, rhinovirus, **west nile virus**, Ebola virus, foot and mouth virus, and papilloma virus.

96. A kit for detecting the presence of a nucleic acid in a sample, comprising the compound of claim 15.

97. A kit for detecting the presence of a target molecule in a sample, comprising the compound of claim 15.

98. A kit for detecting the presence of a nucleic acid in a cancer cell, comprising the compound of any of claim 74.

99. A kit for detecting the presence of a nucleic acid in a virus infected cell, comprising the compound of claim 74.

100. The compound of any of claims 2, 3, 4, or 7, wherein said compound contains a modified phosphate.

101. The compound of any of claims 1, 2, 3, 4, 6, or 7, wherein said phosphorus containing group is a phosphoramidite, phosphodiester, phosphoramidate, phosphorothioate, phosphorodithioate, alkylphosphonate, arylphosphonate, monophosphate, diphosphate, triphosphate, or pyrophosphate.

102. The compound of claim 12, wherein said nucleoside is carbovir or abacavir.

103. A method for synthesizing a compound having Formula XVIII: ##STR71## wherein each R_6 and R_7 is independently hydrogen,

alkyl or nitrogen protecting group, comprising; (a) treating folic acid with a carboxypeptidase to yield a compound of Formula XIX; ##STR72## (b) introducing protection of the secondary amine to yield a compound of Formula XX; ##STR73## wherein R_6 is a nitrogen protecting group; and (c) introducing protection of the primary amine to yield a compound of Formula XVIII.

104. The method of claim 103, wherein R_6 is trifluoroacetyl (TFA).

105. The method of claim 103, wherein R_7 is isobutyryl (iBu).

106. The method of claim 9, wherein said amino protected pteroic acid is a compound of Formula XVIII.

107. The method of claim 10, wherein said amino protected pteroic acid is a compound of Formula XVIII.

108. A compound of claim 1, having Formula XXI: ##STR74## wherein each "n" is independently an integer from 0 to about 200.

109. A compound of claim 4, having Formula XXII: ##STR75## wherein each "n" is independently an integer from 0 to about 200.

110. A compound of claim 7, having Formula XXIII: ##STR76## wherein "n" is an integer from 0 to about 200.

111. A compound having Formula XXIV: ##STR77## wherein "n" is an integer from 0 to about 200.

112. A compound having Formula XXV: ##STR78## wherein each R_5 and R_7 is independently hydrogen, alkyl or a nitrogen protecting group, each R_{15}, R_{16}, R_{17}, and R_{18} is independently O, S, alkyl, substituted alkyl, aryl, substituted aryl, or halogen, X_1 is --CH(X_1') or a group of Formula XXVI: ##STR79## wherein R_4 is a protecting group and "n" is an integer from 0 to about 200; X_1' is the protected or unprotected side chain of a naturally occurring or non-naturally-occurring amino acid, X_2 is an amide, alkyl, or carbonyl containing linker or a bond, and X_3 is a degradable linker which is optionally absent.

113. The compound of claim 112, wherein X_3 is a group of Formula XXVI: ##STR80## wherein R_4 is hydrogen or a protecting group, "n" is an integer from 0 to about 200 and R_{12} is a straight or branched chain alkyl, substituted alkyl, aryl, or substituted aryl.

114. The compound of claim 113, wherein R_4 is hydrogen and R_{12} is methyl or hyrdogen.

115. A pharmaceutical composition comprising the compound of claim 108 in a pharmaceutically acceptable carrier.

116. A pharmaceutical composition comprising the compound of claim 109 in a pharmaceutically acceptable carrier.

117. A pharmaceutical composition comprising the compound of claim 110 in a pharmaceutically acceptable carrier.

118. A pharmaceutical composition comprising the compound of claim 111 in a pharmaceutically acceptable carrier.

119. A pharmaceutical composition comprising the compound of claim 112 in a pharmaceutically acceptable carrier.

120. A method of treating a cancer patient, comprising contacting cells of said patient with the pharmaceutical composition of any of claims 115-119, under conditions suitable for said treatment.

121. The method of claim 120, further comprising the use of one or more other therapies under conditions suitable for said treatment.

122. The method of claim 120, wherein said cancer is breast cancer, lung cancer, colorectal cancer, brain cancer, esophageal cancer, stomach cancer, bladder cancer, pancreatic cancer, cervical cancer, head and neck cancer, ovarian cancer, melanoma, lymphoma, glioma, or multidrug resistant cancers.

123. The compound of claim 2, wherein R_{12} is a alkylhyrdroxyl.

124. The compound of claim 123, wherein said alkylhydroxyl is $--(CH_2)_{nOH}$.

125. The compound of claim 124, wherein said n is an integer from 1-10.

126. The kit of claim 96, wherein said sample is from a cancer cell.

127. The kit of claim 96, wherein said sample is from a virus infected cell.

128. A compound having Formula XXVII: ##STR81## wherein "n" is an integer from about 0 to about 20 and R_4 is H or a cationic salt.

129. A method for synthesizing a compound having Formula XXVII comprising: (a) Selective tritylation of the thiol of cysteamine under conditions suitable to yield a compound having Formula XXVIII: ##STR82## wherein "n" is an integer from about 0 to about 20 and R_{19} is a thiol protecting group; (b) peptide coupling of the product of (a) with a compound having Formula XXIX: ##STR83## wherein R_{20} is a carboxylic acid protecting group and R_{21} is an amino protecting group, under conditions suitable to yield a compound having Formula XXX: ##STR84## wherein "n" is an integer from about 0 to about 20, R_{19} is a thiol protecting group, R_{20} is a carboxylic acid protecting group and R_{21} is an amino protecting group; (c) removing the amino protecting group R_{21} of the product of (b) under conditions suitable to yield a compound having Formula XXXI: ##STR85## wherein "n" is an integer from about 0 to about 20 and R_{19} and R_{20} are as described in (b); (d) condensation of the product of (c) with a compound having Formula XXXII: ##STR86## wherein R_{22} is an amino protecting group, under conditions suitable to yield a compound having Formula XXXIII: ##STR87## wherein "n" is an integer from about 0 to about 20 and R_{19} and R_{20} are as described in (b) and R_{22} is as described in (d); (e) selective cleave of R_{22} from the product of (d) under conditions suitable to yield a compound having Formula XXXIV: ##STR88## wherein "n" is an integer from about 0 to about 20 and R_{19} and R_{20} are as described in (b); (f) coupling the product of (e) with a compound having Formula XXXV: ##STR89## wherein R_{23} is an amino protecting group under conditions suitable to yield a compound having Formula XXXVI: ##STR90## wherein R_{23} is an amino protecting group, "n" is an integer from about 0 to about 20 and R_{19} and R_{20} are as described in (b); (g) deprotecting the product of (f) under conditions suitable to yield a compound having Formula XXVIII. ##STR91## wherein "n" is an integer from about 0 to about 20; and (h) introducing a disulphide-based leaving group to the product of (g) under conditions suitable to yield a compound having Formula XXVII.

130. A compound having Formula XXIX: ##STR92## wherein "n" is an integer from about 0 to about 20, X is a nucleic acid, polynucleotide, or oligonucleotide, and P is a phosphorus containing group.

131. A method for synthesizing a compound having Formula XXIX, comprising: (a) Coupling a thiol containing linker to a nucleic acid, polynucleotide or oligonucleotide under conditions suitable to yield a compound having Formula XXX: ##STR93## wherein "n" is an integer from about 0 to about 20, X is a nucleic acid, polynucleotide, or oligonucleotide, and P is a phosphorus containing group; and (b) coupling the product of (a) with a compound having Formula XXVII under conditions suitable to yield a compound having Formula XXIX.

132. The method of claim 131, wherein said thiol containing linker is a compound having Formula XXXI: ##STR94## wherein "n" is an integer from about 0 to about 20, P is a phosphorus containing group, and R24 is any alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, alkenyl, substituted alkenyl, alkynyl, or substituted alkynyl group with or without additional protecting groups.

133. The method of claim 131, wherein said conditions suitable to yield a compound having Formula XXX comprises reduction of the disulfide bond of a compound having Formula XXXII: ##STR95## wherein "n" is an integer from about 0 to about 20, X is a nucleic acid, polynucleotide, or oligonucleotide, P is a phosphorus containing group, and R_{24} is any alkyl, substituted alkyl, alkoxy, aryl, substituted aryl, alkenyl, substituted alkenyl, alkynyl, or substituted alkynyl group with or without additional protecting groups.

134. A pharmaceutical composition comprising the compound of claim 128 in a pharmaceutically acceptable carrier.

135. A pharmaceutical composition comprising the compound of claim 130 in a pharmaceutically acceptable carrier.

136. A method of treating a cancer patient, comprising contacting cells of said patient with the pharmaceutical composition of any claim 134 or claim 135, under conditions suitable for said treatment.

137. The kit of claim 97, wherein said sample is from a cancer cell.

138. The kit of claim 97, wherein said sample is from a virus infected cell.

L14 ANSWER 24 OF 34 USPATFULL on STN
2003:134060 Viral vaccine composition, process, and methods of use.
Jira, Vic, El Monte, CA, UNITED STATES
Jirathitikal, Vichai, Chachoengsao, THAILAND
US 2003092145 A1 20030515
APPLICATION: US 2001-935344 A1 20010823 (9)
PRIORITY: US 2000-227520P 20000824 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A composition for treating or preventing virus-induced infections is described, along with a process of producing the composition and methods of the composition's use. The composition comprises viral pathogen-infected cell or tissue, or malignantly or immunologically aberrant cells or tissues which has been reduced and/or denatured. The preferred composition is administered across a mucosal surface of an animal suffering or about suffer from infection. The composition is administered as preventive or therapeutic vaccine.

CLM What is claimed is:

1. A multivalent antiviral vaccine comprising one or more heat-inactivated viral antigens, wherein at least one viral antigen is capable in producing an immune response in a host when said vaccine is administered orally at a dose that is sufficient for preventing or treating the viral disease in said host.

2. The viral antigen of claim 1 wherein said antigen is derived from influenza virus, cytomegalovirus, avian leukosis-sarcoma virus, Rous Sarcoma virus, Mammalian C-type Murine leukemia virus, Feline leukemia virus, simian sarcoma virus, B-type Mouse mammary tumor virus, D-type virus Mason-Pfizer monkey virus, simian AIDS virus, Human T-cell leukemia virus, Simian T-cell leukemia virus, bovine leukemia virus, Human immunodeficiency virus, Simian immunodeficiency virus, Feline immunodeficiency virus, Visna/maedi virus, Equine infectious anemia virus, Caprine arthritis-encephalitis virus, spumavirus, foamy virus, endogenous retrovirus, papilloma virus, respiratory syncytial virus, poliomyelitis virus, pox virus, measles virus, arbor virus, Coxsackie virus, herpes virus, hantavirus, hepatitis virus, baculovirus, mumps virus, circovirus, arenavirus, rotavirus, Colorado Tick Fever CTF virus, Eyach virus, Langat virus, Powassan virus, Omsk hemorrhagic fever virus, Crimean-Congo hemorrhagic fever virus, Yellow fever virus, Encephalitis virus, St. Louis Encephalitis virus, Venezualan equine encephalitis virus, Western equine encephalitis virus, Chikungunya virus, Japanese encephalitis virus, **West Nile virus**, Kyasanur forest disease virus, Dengue fever virus, California encephalitis virus, adenovirus, Korean haemorrhagic fever virus, hantavirus, Argentine haemorrhagic fever virus, Junin virus, Aujeszky disease virus, Pseudorabies virus, Herpesvirus, Chikungunya virus, cowpox virus, ebolavirus, Ganjam virus, herpesvirus simiae, Lassa fever virus, Louping ill virus, Lymphocytic choriomeningitis virus, Marburg virus, Milkers nodule virus, Newcastle disease virus, Omsk haemorrhagic fever virus, Orf virus, Parvovirus, Poliovirus, Pseudorabies, Rabies virus, Rift Valley fever virus, Russian Spring-Summer encephalitis virus, Sabia virus, vaccinia virus, vesicular stomatitis virus, Western equine encephalitis virus, or Yelllow fever virus.

3. A composition for the induction of immunity to a viral pathogen in a host in need thereof, said composition comprising reduced viral pathogen formulated as an oral pill.

4. The viral pathogen of claim 3 wherein said pathogen is selected from a group consisting of influenza virus, cytomegalovirus, avian leukosis-sarcoma virus, Rous Sarcoma virus, Mammalian C-type Murine leukemia virus, Feline leukemia virus, simian sarcoma virus, B-type Mouse mammary tumor virus, D-type virus Mason-Pfizer monkey virus, simian AIDS virus, Human T-cell leukemia virus, Simian T-cell leukemia virus, bovine leukemia virus, Human immunodeficiency virus, Simian

immunodeficiency virus, Feline immunodeficiency virus, Visna/maedi virus, Equine infectious anemia virus, Caprine arthritis-encephalitis virus, spumavirus, foamy virus, endogenous retrovirus, papilloma virus, respiratory syncytial virus, poliomyelitis virus, pox virus, measles virus, arbor virus, Coxsackie virus, herpes virus, hantavirus, hepatitis virus, baculovirus, mumps virus, circovirus, arenavirus, rotavirus, Colorado Tick Fever CTF virus, Eyach virus, Langat virus, Powassan virus, Omsk hemorrhagic fever virus, Crimean-Congo hemorrhagic fever virus, Yellow fever virus, Encephalitis virus, St. Louis Encephalitis virus, Venezualan equine encephalitis virus, Western equine encephalitis virus, Chikungunya virus, Japanese encephalitis virus, **West Nile virus**, Kyasanur forest disease virus, Dengue fever virus, California encephalitis virus, adenovirus, Korean haemorrhagic fever virus, hantavirus, Argentine haemorrhagic fever virus, Junin virus, Aujeszky disease virus, Pseudorabies virus, Herpesvirus, Chikungunya virus, cowpox virus, ebolavirus, Ganjam virus, herpesvirus simiae, Lassa fever virus, Louping ill virus, Lymphocytic choriomeningitis virus, Marburg virus, Milkers nodule virus, Newcastle disease virus, Omsk haemorrhagic fever virus, Orf virus, Parvovirus, Poliovirus, Pseudorabies, Rabies virus, Rift Valley fever virus, Russian Spring-Summer encephalitis virus, Sabia virus, vaccinia virus, vesicular stomatitis virus, Western equine encephalitis virus, and Yelllow fever virus.

5. An immunogen formulated as an oral pill, wherein upon oral administration, said immunogen retains the ability to elicit an immune response in a host in need of immune response.

6. The immunogen of claim 5 is administered to the host without an immune adjuvant.

7. The immunogen of claim 5 wherein said immunogen is selected from a group consisting of immunogens derived from fungi influenza virus, cytomegalovirus, avian leukosis-sarcoma virus, Rous Sarcoma virus, Mammalian C-type Murine leukemia virus, Feline leukemia virus, simian sarcoma virus, B-type Mouse mammary tumor virus, D-type virus Mason-Pfizer monkey virus, simian AIDS virus, Human T-cell leukemia virus, Simian T-cell leukemia virus, bovine leukemia virus, Human immunodeficiency virus, Simian immunodeficiency virus, Feline immunodeficiency virus, Visna/maedi virus, Equine infectious anemia virus, Caprine arthritis-encephalitis virus, spumavirus, foamy virus, endogenous retrovirus, papilloma virus, respiratory syncytial virus, poliomyelitis virus, pox virus, measles virus, arbor virus, Coxsackie virus, herpes virus, hantavirus, hepatitis virus, baculovirus, mumps virus, circovirus, arenavirus, rotavirus, Colorado Tick Fever CTF virus, Eyach virus, Langat virus, Powassan virus, Omsk hemorrhagic fever virus, Crimean-Congo hemorrhagic fever virus, Yellow fever virus, Encephalitis virus, St. Louis Encephalitis virus, Venezualan equine encephalitis virus, Western equine encephalitis virus, Chikungunya virus, Japanese encephalitis virus, **West Nile virus**, Kyasanur forest disease virus, Dengue fever virus, California encephalitis virus, adenovirus, Korean haemorrhagic fever virus, hantavirus, Argentine haemorrhagic fever virus, Junin virus, Aujeszky disease virus, Pseudorabies virus, Herpesvirus, Chikungunya virus, cowpox virus, ebolavirus, Ganjam virus, herpesvirus simiae, Lassa fever virus, Louping ill virus, Lymphocytic choriomeningitis virus, Marburg virus, Milkers nodule virus, Newcastle disease virus, Omsk haemorrhagic fever virus, Orf virus, Parvovirus, Poliovirus, Pseudorabies, Rabies virus, Rift Valley fever virus, Russian Spring-Summer encephalitis virus, Sabia virus, vaccinia virus, vesicular stomatitis virus, Western equine encephalitis virus, and Yelllow fever virus.

8. The immune response of claim 5 wherein said immune response is directed against viral infection.

9. The oral pill of claim 5 wherein the amount of immunogen comprised in said pill is between about 0.0000001% to about 20% by weight.

10. An oral composition suitable for treating or preventing a pathogen-induced infection in a host, the composition comprising a first component comprising a denatured antigen of an infection-inducing pathogen and a second component comprising a denatured tissue from a pathogen-infected host.

11. The oral composition of claim 10 said composition further comprising calcium.

12. The oral composition of claim 11 said composition further comprising magnesium.

L14 ANSWER 25 OF 34 USPATFULL on STN
2003:133510 West nile vaccine.
Chu, Hsien-Jue, Fort Dodge, IA, UNITED STATES
Wyeth, Madison, NJ (U.S. corporation)
US 2003091595 A1 20030515
APPLICATION: US 2002-202716 A1 20020725 (10)
PRIORITY: US 2001-308334P 20010727 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides a safe and effective vaccine composition against **West Nile virus** disease. An immunogenically active component of **West Nile virus** or plasmid DNA, an adjuvant such as a metabolizable oil, and a pharmacologically acceptable carrier are formulated into an immunizing vaccine. The invention also provides a method for the prevention or amelioration of West Nile disease, such as encephalitis, in equidae by administering the vaccine composition herein set forth.

CLM What is claimed is:

1. A safened vaccine composition which comprises: an effective immunizing amount of an immunogenically active component selected from the group consisting of an inactivated whole or subunit **West Nile virus**, an antigen derived from said virus, DNA derived from said virus, and a mixture thereof; an immunogenically stimulating amount of a metabolizable oil; and a pharmacologically acceptable carrier.

2. The composition according to claim 1 wherein the immunogenically active component is an inactivated whole or subunit **West Nile virus**, and further wherein a dosage unit of said vaccine comprises about 1×10^4 $TCID_{50}$ to about 1×10^8 $TCID_{50}$ of said virus.

3. The composition according to claim 1 wherein the metabolizable oil is SP oil.

4. The composition according to claim 2 wherein the immunogenically active component is an inactivated whole **West Nile virus**.

5. The composition according to claim 3 wherein said oil is present in the amount of about 4% to 10% vol/vol.

6. The composition according to claim 4 wherein said virus is present in sufficient quantity to provide at least about 1×10^4 $TCID_{50}$ per unit dose of said composition.

7. The composition according to claim 5 wherein said oil is present in the amount of about 5% vol/vol.

8. The composition according to claim 7 wherein said virus is present in sufficient quantity to provide at least about 1×10^6 $TCID_{50}$ per unit dose.

9. The composition according to claim 8 wherein said virus is inactivated whole **West Nile virus**.

10. A method for the prevention or amelioration of West Nile encephalitis in equidae which comprises administering to said equidae a safened vaccine composition which comprises an effective immunizing amount of an immunogenically active component selected from the group consisting of an inactivated whole or subunit **West Nile virus**, an antigen derived from said virus, DNA derived from said virus, and a mixture thereof; an immunogenically stimulating amount of a metabolizable oil; and a pharmacologically acceptable carrier.

11. The method according to claim 10 having said vaccine composition wherein the immunogenically active component is an inactivated whole or subunit **West Nile virus**.

12. The method according to claim 10 having said vaccine composition wherein the metabolizable oil is SP oil.

13. The method according to claim 10 wherein said equidae are horses.

14. The method according to claim 13 wherein said horses are pregnant mares.

15. The method according to claim 10 wherein said vaccine composition is administered parenterally.

16. The method according to claim 10 wherein said vaccine composition is administered intramuscularly.

17. The method according to claim 12 having a vaccine composition wherein the immunogenically active component is inactivated whole **West Nile virus**.

18. The method according to claim 17 wherein said virus is present in an amount sufficient to provide at least about 1×10^4 $TCID_{50}$ and up to about 1×10^9 $TCID_{50}$ per unit dose.

19. The method according to claim 18 wherein said virus is present in an amount sufficient to provide at least about 1×10^6 $TCID_{50}$ per unit dose, and at least two doses are administered to each said equidae.

20. The method according to claim 19 wherein the metabolizable oil is SP oil and is present in an amount of about 5% vol/vol.

21. The composition according to claim 6 further comprising another vaccine component directed against rabies virus, Eastern equine encephalitis virus, Western equine encephalitis virus, Venezuelan equine encephalitis virus, equine herpes virus such as EHV-1 or EHV-4, Ehrlichia risticii, Streptococcus equi, tetanus toxoid, equine influenza virus (EIV).

22. A vaccine composition, comprising: a) at least about 1×10^4 $TCID_{50}$ and up to about 1×10^9 $TCID_{50}$ per unit dose of inactivated **West Nile virus**; and b) about 4% to 10% vol/vol of a metabolizable oil adjuvant comprising about 1 to 3% polyoxyethylene-polyoxypropylene block copolymer, about 2 to 6% of squalane and about 0.1 to 0.5% of polyoxyethylene sorbitan monooleate.

23. A safened and effective **West Nile virus** vaccine for equidae, comprising: a) at least about 1×10^6 $TCID_{50}$ to about 1×10^8 $TCID_{50}$ per unit dose of killed or inactivated **West Nile virus**, and b) at least about 1% vol/vol of an adjuvant comprising at least one metabolizable oil and at least one wetting or dispersing agent.

24. The vaccine of claim 23, wherein said vaccine comprises at least about 1×10^7 $TCID_{50}$ of said virus.

25. The vaccine of claim 24, wherein said vaccine comprises at least about 5×10^7 $TCID_{50}$ of said virus.

26. The vaccine of claim 23, wherein said vaccine is formulated into two dosage units.

27. The vaccine of claim 23, comprising at least about 4% of said adjuvant.

28. The vaccine of claim 27, comprising about 4% to 10% of said adjuvant.

29. The vaccine of claim 23, wherein said adjuvant is SP oil.

30. The vaccine of claim 23, comprising at least two wetting or dispersing agents.

31. The vaccine of claim 30, wherein said wetting or dispersing agents are selected from the group consisting of non-ionic surfactants.

32. The vaccine of claim 31, wherein said non-ionic surfactants are selected from the group consisting of polyoxyethylene/polyoxypropylene block copolymers and polyoxyethylene esters.

33. A vaccine regimen for horses or other equidae, comprising two dosage units of killed or inactivated **West Nile virus**, wherein each said dosage unit comprises about 0.5 to 5 milliliters of a composition containing at least about 5×10^7 $TCID_{50}$ of said virus and about 1 to 10% vol/vol of an adjuvant, said adjuvant comprising at least one metabolizable oil and at least two nonionic surfactants, and further wherein said dosage unit comprises a pharmacologically acceptable carrier.

34. The vaccine regimen of claim 33, wherein said adjuvant is SP oil.

35. The vaccine regimen of claim 34, wherein said SP oil comprises squalane in an amount of about 2 to 6% by weight of said adjuvant, about 1 to 3% of polyoxyethylene/polyoxypropylene block copolymer by weight of said adjuvant, and about 0.1 to 0.5% of polyoxyethylene sorbitan monooleate by weight of said adjuvant.

36. The vaccine regimen of claim 33, further comprising a vaccine component directed to at least one member selected from the group consisting of influenza virus, Eastern, Western and Venezuelan rhinopneumonitis virus and tetanus toxoid.

37. A **West Nile virus** vaccine composition comprising West Nile plasmid DNA in an amount of about 50 to 3,000 micrograms per dose.

38. The composition of claim 37, wherein said composition comprises about 100 to 1,000 micrograms per dose.

39. The composition of claim 38, wherein said composition comprises about 100 to 250 micrograms per dose.

40. A **West Nile virus** vaccine regimen which comprises administering to a horse at least about one dose of the composition of claim 37.

41. The regimen of claim 40, which comprises administering at least about two doses.

L14 ANSWER 26 OF 34 USPATFULL on STN
2003:92716 Recombinant gelatins in vaccines.
Chang, Robert C., Burlingame, CA, UNITED STATES
Kivirikko, Kari I., Oulu, FINLAND
Neff, Thomas B., Atherton, CA, UNITED STATES
Olsen, David R., Menlo Park, CA, UNITED STATES
Polarek, James W., Sausalito, CA, UNITED STATES
US 2003064074 A1 20030403
APPLICATION: US 2002-232175 A1 20020830 (10)
PRIORITY: US 2000-204437P 20000515 (60)
US 1999-165114P 19991112 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to vaccines comprising recombinant gelatin, to methods of producing and using such vaccines, and to vaccination kits.
CLM What is claimed is:
1. A vaccine composition comprising: (a) a recombinant gelatin; and (b) an antigenic agent.

2. The vaccine composition of claim 1, wherein the recombinant gelatin is recombinant human gelatin.

3. The vaccine composition of claim 1, wherein the recombinant gelatin comprises a homogeneous mixture of recombinant gelatin polypeptides.

4. The vaccine composition of claim 1, wherein the recombinant gelatin comprises a heterogeneous mixture of recombinant gelatin polypeptides.

5. The vaccine composition of claim 1, wherein the recombinant gelatin is non- hydroxylated.

6. The vaccine composition of claim 1, wherein the recombinant gelatin is hydroyxlated.

7. The vaccine composition of claim 1, wherein the recombinant gelatin has a percentage hydroxylation selected from the group consisting of 20 to 80%, 30 to 80%, 40 to 80%, 60 to 80%, 80 to 100%, 20 to 60%, 30 to 60%, 40 to 60%, 20 to 30%, 20 to 40%, and 30 to 40%.

8. The vaccine composition of claim 1, wherein the recombinant gelatin is hydrolyzed.

9. The vaccine composition of claim 1, wherein the recombinant gelatin is derived from non-native collagen sequence.

10. The vaccine composition of claim 1, wherein the recombinant gelatin is obtained from one type of collagen free of any other type of collagen.

11. The vaccine composition of claim 1, wherein the recombinant gelatin is proteolytically stable.

12. The vaccine composition of claim 1, wherein the recombinant gelatin is produced by processing of recombinant collagen.

13. The vaccine composition of claim 1, wherein the recombinant gelatin is produced directly from an altered collagen construct.

14. The vaccine composition of claim 1, wherein the recombinant gelatin has a molecular weight range selected from the group consisting of about 0 to 50 kDa, about 10 to 30 kDa, about 30 to 50 kDa, about 10 to 70 kDa, about 50 kDa to 70 kDa about 50 to 100 kDa, about 100 to 150 kDa, about 150 to 200 kDa, about 200 to 250 kDa, about 250 to 300 kDa, and about 300 to 350 kDa.

15. The vaccine composition of claim 1, wherein the recombinant gelatin has a molecular weight selected from the group consisting of about 1 kDa, about 5 kDa, about 8 kDa, about 9 kDa, about 14 kDa, about 16 kDa, about 22 kDa, about 23 kDa, about 44 kDa, and about 65 kDa.

16. The vaccine composition of claim 1, wherein the recombinant gelatin comprises a sequence selected from the group consisting of SEQ ID NOs: 15 through 25, and 30, 31, and 33.

17. The vaccine composition of claim 1, wherein the recombinant gelatin is non-immunogenic.

18. The vaccine composition of claim 1, wherein the recombinant gelatin confers stability at ambient temperatures.

19. The vaccine composition of claim 1, wherein the vaccine composition is suitable for injectable delivery.

20. The vaccine composition of claim 1, wherein the vaccine composition is suitable for nasal delivery.

21. The vaccine composition of claim 1, wherein the vaccine composition is suitable for oral delivery.

22. The vaccine composition of claim 1, wherein the vaccine composition is suitable for transdermal delivery.

23. The vaccine composition of claim 1, wherein the vaccine composition is suitable for mucosal delivery.

24. The vaccine composition of claim 1, wherein the vaccine composition is suitable for deep lung delivery.

25. The vaccine composition of claim 1, wherein the vaccine composition is liquid.

26. The vaccine composition of claim 1, wherein the vaccine composition is dry.

27. The vaccine composition of claim 1, wherein the vaccine composition is lyophilized.

28. The vaccine composition of claim 1, wherein the vaccine composition is powdered.

29. The vaccine composition of claim 1, wherein the vaccine composition is a spray.

30. The vaccine composition of claim 1, wherein the vaccine composition is an inhalant.

31. The vaccine composition of claim 1, wherein the vaccine composition comprises a live vaccine.

32. The vaccine composition of claim 1, wherein the vaccine composition comprises an attenuated vaccine.

33. The vaccine composition of claim 1, wherein the vaccine composition comprises an inactivated vaccine.

34. The vaccine composition of claim 1, wherein the vaccine composition comprises a subunit vaccine.

35. The vaccine composition of claim 1, wherein the vaccine composition comprises a single dosage.

36. The vaccine composition of claim 1, wherein the vaccine composition comprises a multiple dosage.

37. The vaccine composition of claim 1, wherein the vaccine composition comprises a conjugate vaccine.

38. The vaccine composition of claim 1, wherein the vaccine composition comprises a nucleic acid vaccine.

39. The vaccine composition of claim 38, wherein the nucleic acid vaccine is a DNA vaccine.

40. The vaccine composition of claim 1, wherein the vaccine composition is a combined vaccine.

41. The vaccine composition of claim 1, wherein the vaccine composition comprises an acellular vaccine.

42. The vaccine composition of claim 1, wherein the vaccine composition comprises a vaccine formulated for the prevention of a disease selected from the group consisting of vacinnia virus (small pox), polio virus (Salk and Sabin), mumps, measles, rubella, diphtheria, tetanus, Varicella-Zoster (chicken pox/shingles), pertussis (whopping cough), Bacille Calmette-Guerin (BCG, tuberculosis), haemophilus influenzae meningitis, rabies, cholera, Japanese encephalitis virus, salmonella typhi, shigella, hepatitis A, hepatitis B, adenovirus, yellow fever, foot-and-mouth disease, herpes simplex virus, respiratory syncytial virus, rotavirus, Dengue, **West Nile virus**, Turkey herpes virus (Marek's Disease), influenza, and anthrax.

43. The vaccine composition of claim 1, wherein the recombinant gelatin has an endotoxin level of below 1.000 EU/mg.

44. The vaccine composition of claim 1, wherein the recombinant gelatin has an endotoxin level of below 0.500 EU/mg.

45. The vaccine composition of claim 1, wherein the recombinant gelatin has an endotoxin level of below 0.050 EU/mg.

46. The vaccine composition of claim 1, wherein the recombinant gelatin has an endotoxin level of below 0.005 EU/mg.

47. A method of producing a vaccine composition comprising recombinant gelatin, the method comprising: (a) providing an antigenic agent; (b) providing a recombinant gelatin; and (c) combining the antigenic agent and the recombinant gelatin.

48. A vaccine stabilizer comprising a recombinant gelatin.

49. A vaccine stabilizer comprising a recombinant human gelatin.

50. A method of inducing an immune response in a subject, the method comprising administering the vaccine composition of claim 1 to the subject.

51. A method of stabilizing a vaccine composition, the method comprising adding the vaccine stabilizer of claim 48 to a vaccine composition.

52. A vaccination kit, the kit comprising: (a) a vaccine comprising recombinant gelatin; and (b) a delivery device for delivery of the vaccine.

L14 ANSWER 27 OF 34 USPATFULL on STN
2003:30900 Nucleic acid vaccines for prevention of flavivirus infection.
Chang, Gwong-Jen J., Fort Collins, CO, UNITED STATES
US 2003022849 A1 20030130
APPLICATION: US 2001-826115 A1 20010404 (9)
PRIORITY: US 1998-87908P 19980604 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention encompasses isolated nucleic acids containing transcriptional units which encode a signal sequence of one flavivirus and an immunogenic flavivirus antigen of a second flavivirus. The invention further encompasses a nucleic acid and protein vaccine and the use of the vaccine to immunize a subject against flavivirus infection.

The invention also provides antigens encoded by nucleic acids of the invention, antibodies elicited in response to the antigens and use of the antigens and/or antibodies in detecting flavivirus or diagnosing flavivirus infection.

CLM What is claimed is:

1. An isolated nucleic acid comprising a transcriptional unit encoding a signal sequence of a structural protein of a first flavivirus and an immunogenic flavivirus antigen of a second flavivirus, wherein the transcriptional unit directs the synthesis of the antigen.

2. The nucleic acid of claim 1, wherein the signal sequence is a Japanese encephalitis virus signal sequence.

3. The nucleic acid of claim 1, wherein the immunogenic flavivirus antigen is of a flavivirus selected from the group consisting of yellow fever virus, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, Powassan virus and **West Nile virus**.

4. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of **West Nile virus**.

5. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of yellow fever virus.

6. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of St. Louis encephalitis virus.

7. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of Powassan virus.

8. The nucleic acid of claim 1, wherein the antigen is selected from the group consisting of an M protein of a flavivirus, an E protein of a flavivirus, both an M protein and an E protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an E protein of a flavivirus and both a portion of an M protein of a flavivirus and a portion of an E protein of a flavivirus or any combination thereof.

9. The nucleic acid of claim 8, wherein the antigen is both the M protein and the E protein of a flavivirus.

10. The nucleic acid of claim 1, wherein the nucleic acid is DNA.

11. The nucleic acid of claim 10, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:19, SEQ ID NO:21 and SEQ ID NO:23.

12. The nucleic acid of claim 1, wherein the transcriptional unit comprises a control sequence disposed appropriately such that it operably controls the synthesis of the antigen.

13. The nucleic acid of claim 12, wherein the control sequence is the cytomegalovirus immediate early promoter.

14. The nucleic acid of claim 1, comprising a Kozak consensus sequence located at a translational start site for a polypeptide comprising the antigen encoded by the TU.

15. The nucleic acid of claim 1 wherein the transcriptional unit comprises a poly-A terminator.

16. A cell comprising the nucleic acid of claim 1.

17. A composition comprising the nucleic acid of claim 1 and a pharmaceutically acceptable carrier.

18. A method of immunizing a subject against infection by a flavivirus, comprising administering to the subject an effective amount of the composition of claim 17.

19. The method of claim 18, wherein the flavivirus antigen is of a flavivirus selected from the group consisting of yellow fever virus, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, Powassan virus and **West Nile virus**.

20. The method of claim 18, wherein the antigen is selected from the group consisting of an M protein of a flavivirus, an E protein of a flavivirus, both an M protein and an E protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an E protein of a flavivirus and both a portion of an M protein of a flavivirus and a portion of an E protein of a flavivirus or any combination thereof.

21. The method of claim 20, wherein the antigen is both the M protein and the E protein of a flavivirus, and wherein a cell within the body of the subject, after incorporating the nucleic acid within it, secretes subviral particles comprising the M protein and the E protein.

22. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of **West Nile virus**.

23. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of yellow fever virus.

24. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of St. Louis encephalitis virus.

25. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of Powassan virus.

26. The method of claim 18, comprising administering the composition to the subject in a single dose.

27. The method of claim 18, wherein the composition is administered via a parenteral route.

28. The nucleic acid of claim 1, wherein the antigen is a St. Louis encephalitis virus antigen.

29. The method of claim 18, wherein the antigen is a St. Louis encephalitis virus antigen.

30. The nucleic acid of claim 1, wherein the antigen is a Japanese encephalitis virus antigen.

31. The method of claim 18, wherein the antigen is a Japanese encephalitis virus antigen.

32. The nucleic acid of claim 1, wherein the antigen is a yellow fever virus antigen.

33. The method of claim 18, wherein the antigen is a yellow fever virus antigen.

34. The nucleic acid of claim 1, wherein the antigen is a dengue virus antigen.

35. The method of claim 18, wherein the antigen is a dengue virus antigen.

36. The nucleic acid of claim 1, wherein the antigen is a **West Nile virus** antigen.

37. The method of claim 18, wherein the antigen is a **West Nile virus** antigen.

38. An antigen produced from the nucleic acid of claim 1.

39. A method of detecting a flavivirus antibody in a sample, comprising: (a) contacting the sample with the antigen of claim 38 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby detecting a flavivirus antibody in the sample.

40. An antibody produced in response to immunization by the antigen of claim 38.

41. A method of detecting a flavivirus antigen in a sample, comprising: (a) contacting the sample with the antibody of claim 40 under conditions whereby an antigen/antibody complex can form; and (b) detecting

antigen/antibody complex formation, thereby detecting a flavivirus antigen in a sample.

42. A method of diagnosing a flavivirus infection in a subject, comprising: (a) contacting a sample from the subject with the antigen of claim 38 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby diagnosing a flavivirus infection in a subject.

43. A method of diagnosing a flavivirus infection in a subject, comprising: (a) contacting a sample from the subject with the antibody of claim 40 under conditions whereby an antigen/antibody complex can form; and (b) detecting antigen/antibody complex formation, thereby diagnosing a flavivirus infection in a subject.

L14 ANSWER 28 OF 34 USPATFULL on STN
2003:30880 Novel antiviral activities primate theta defensins and mammalian cathelicidins.
Maury, Wendy, Coralville, IA, UNITED STATES
Stapleton, Jack, Iowa City, IA, UNITED STATES
Roller, Richard, Coralville, IA, UNITED STATES
Stinski, Mark, North Liberty, IA, UNITED STATES
McCray, Paul B., Iowa City, IA, UNITED STATES
Tack, Brian, Iowa City, IA, UNITED STATES
US 2003022829 A1 20030130
APPLICATION: US 2002-60102 A1 20020129 (10)
PRIORITY: US 2001-265270P 20010130 (60)
US 2001-309368P 20010801 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to the use of anti-viral peptides in the inhibition and treatment of viral infections, in particular infections caused by enveloped viruses. These anti-viral peptides, some natural and others artificial, adopt either amphiphilic alpha-helical or a theta structure where the homodimeric or heterodimer peptides are joined by both cysteine bonds and circularization of the peptides. These agents may be used alone or in combination with more traditional anti-viral pharmaceuticals.
CLM What is claimed is:
1. A method for reducing the infectivity of a virus comprising contacting said virus with a first anti-viral peptide, said peptide comprising a theta defensin peptide or amphipathic alpha helical structure in a lipid environment.

2. The method of claim 1, wherein said first anti-viral peptide is a naturally-occurring peptide.

3. The method of claim 2, wherein said naturally-occurring peptide is a cathelicidin.

4. The method of claim 3, wherein said cathelicidin is selected from the group consisting of a mouse cathelicidin, a monkey cathelicidin, a human cathelicidin, and a sheep cathelicidin.

5. The method of claim 1, wherein said first anti-viral peptide is a non-naturally occurring peptide.

6. The method of claim 1, wherein said peptide is about 13 to about 35 residues in length.

7. The method of claim 5, wherein said peptide contains a non-naturally occurring amino acid.

8. The method of claim 1, wherein the virus is an enveloped virus.

9. The method of claim 1, wherein the virus infects humans and is selected from the group consisting of HIV, HSV-1, HSV-2, EBV, varicella zoster virus, CMV, herpesvirus B, HHV6, HHV8, respiratory syncytial virus (RSV), influenza A, B and C viruses, hepatitis A, hepatitis B, hepatitis C, hepatitis G, smallpox, vaccinia virus, Marburg virus, ebola virus, dengue virus, **West Nile virus**, hantavirus, measles virus, mumps virus, rubella virus, rabies virus, yellow fever virus, Japanese encephalitis virus, Murray Valley encephalitis virus, Rocio virus, tick-borne encephalitis virus, St. Louis encephalitis virus, chikungynya virus, o'nyong-nyong virus, Ross River virus, Mayaro virus, human coronaviruses 229-E and OC43, vesicular stomatitis virus, sandfly fever virus, Rift Valley River virus, Lasa virus, lymphocytic choriomeningitis virus, Machupo virus, Junin virus, HTLV-I and -II.

10. The method of claim 1, wherein the virus infects sheep and is selected from the group consisting of border disease virus, Maedi virus, and visna virus.

11. The method of claim 1, wherein the virus infects cattle and is selected from the group consisting of bovine leukemia virus, bovine diarrhea virus, bovine lentivirus, and infectious bovine rhinotracheitis virus.

12. The method of claim 1, wherein the virus infects swine and is selected from the group consisting of swinepox, African swine fever virus, hemagluttinating virus of swine, hog cholera virus, and pseudorabies virus.

13. The method of claim 1, wherein the virus infects horses and is selected from the group consisting of bovine leukemia virus, bovine diarrhea virus, bovine lentivirus, and infectious bovine rhinotracheitis virus.

14. The method of claim 1, wherein the virus infects cats and is selected from the group consisting of feline immunodeficiency virus, feline leukemia virus, and feline infectious peritonitis virus.

15. The method of claim 1, wherein the virus infects fowl and is selected from the group consisting of Marek's disease virus, turkey bluecomb virus, infectious bronchitis virus of fowl, avian reticuloendotheliosis, sarcoma and leukemia viruses.

16. The method of claim 2, wherein the naturally-occurring peptide is selected from the group consisting of SEQ ID NOS: 1, 2, 3, 4, 5, 6 and 7.

17. The method of claim 5, wherein the non-naturally-occurring peptide is selected from the group consisting of SEQ ID NOS: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24.

18. The method of claim 1, further comprising contacting said virus with a second anti-viral agent.

19. The method of claim 18, wherein said second anti-viral agent is a second anti-viral peptide distinct from said first anti-viral peptide.

20. The method of claim 18, wherein said second anti-viral agent is non-peptide pharmaceutical agent.

21. The method of claim 20, wherein said non-peptide pharmaceutical agent is selected from the group consisting of a protease inhibitor, a nucleoside analog, a viral polymerase inhibitor, and a viral integrase inhibitor.

22. The method of claim 1, wherein said first anti-viral peptide is contacted with said virus at a concentration of about 0.1 to about 50 μg per ml.

23. The method of claim 22, wherein said first anti-viral peptide is contacted with said virus at a concentration of about 1 to about 25 μg per ml.

24. The method of claim 23, wherein said first anti-viral peptide is contacted with said virus at a concentration of about 3 to about 10 μg per ml.

25. The method of claim 1, wherein said virus is located in a tissue or fluid sample.

26. The method of claim 25, wherein said tissue or fluid sample is selected from the group of whole blood, platelets, plasma, and packed blood cells.

27. The method of claim 1, wherein said virus is located in a living subject.

28. The method of claim 27, wherein said first anti-viral peptide is administered topically.

29. The method of claim 27, wherein said first anti-viral peptide is administered to a body cavity.

30. The method of claim 27, wherein said first anti-viral peptide is administered to a mucosal membrane.

31. The method of claim 27, wherein said first anti-viral peptide is administered by injection.

32. The method of claim 27, wherein said first anti-viral peptide is administered by inhalation.

33. The method of claim 27, wherein said first anti-viral peptide is administered orally.

34. The method of claim 27, wherein said first anti-viral peptide is administered to a wound site.

35. The method of claim 27, wherein said patient is immunosuppressed.

36. The method of claim 27, wherein said subject is not infected with said virus, and first anti-viral peptide is administered prior to the virus contacting the subject.

37. The method of claim 27, wherein said first anti-viral peptide is administered subsequent to the virus contacting the subject.

38. The method of claim 37, wherein said subject is chronically infected with said virus.

39. The method of claim 37, wherein said subject is latently infected with said virus.

40. The method of claim 37, wherein said subject is acutely infected with said virus.

41. An anti-viral composition comprising a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure or a theta defensin peptide in a lipid environment, and a second anti-viral agent.

42. The composition of claim 41, wherein said second anti-viral agent is a second anti-viral peptide distinct from said first anti-viral peptide.

43. The composition of claim 41, wherein said second anti-viral agent is a non-peptide pharmaceutical agent.

44. The composition of claim 43, wherein said non-peptide pharmaceutical agent is selected from the group consisting of a protease inhibitor, a nucleoside analog, a viral polymerase inhibitor, and a viral integrase inhibitor.

45. The composition of claim 41, formulated for topical administration.

46. The composition of claim 41, formulated for inhalation.

47. The composition of claim 41, formulated for administration to a mucosal membrane.

48. The composition of claim 41, wherein said composition is located in a sterile i.v. bag.

49. The composition of claim 41, wherein said composition is located in a sterile syringe.

50. The composition of claim 41, wherein said composition is located in sterile tubing.

51. An anti-viral composition comprising a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide, and a contraceptive agent.

52. The composition of claim 51, wherein said composition is located in a condom.

53. The composition of claim 51, wherein said composition is formulated for use in a diaphragm.

54. The composition of claim 51, wherein said composition is formulated for intra-vaginal administration.

55. The composition of claim 51, wherein said contraceptive agent is spermicidal agent or a sperm anti-motility agent.

56. A method of rendering a virus-contaminated tissue or fluid sample safe for use comprising contacting said fluid sample with a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

57. A method for reducing the number of infectious virus particles in a population of viruses comprising contacting said virus population with a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

58. A method of protecting a subject from viral infection comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

59. A method for treating a subject with a viral infection comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

60. A method for preventing a recurrent viral infection in a subject harboring a latent virus comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

61. A method for controlling virus spread within a virally-infected subject comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

62. A method for reducing viral burden in a virally-infected subject comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

63. A method for reducing virus shed from a virally-infected subject comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

64. A method for reducing the percentage of virally-infected subjects in a population comprising administering to said population, regardless of viral infection status, a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

65. A method of inducing latency in a virally-infected subject comprising administering to said subject a first anti-viral peptide, said peptide comprising an amphipathic alpha helical structure in a lipid environment or a theta defensin peptide.

66. The method of claim 1, wherein said first anti-viral peptide is encoded by a nucleic acid that is contained in an expression construct under the control of a promoter active in eukaryotic cells, wherein said expression construct is delivered into a host cell, and said cell supports production and secretion of said first anti-viral peptide which contacts said virus.

67. The method of claim 66, wherein said expression construct is an adenovirus.

68. The method of claim 66, wherein said host cell is infected by said virus.

69. The method of claim 66, wherein said nucleic acid further encodes an intracellular targeting signal fused to said first anti-viral peptide.

70. The method of claim 69, wherein said intracellular targeting signal targets said peptide to one or more of the endoplasmic reticulum, the Golgi apparatus and/or the cell surface.

L14 ANSWER 29 OF 34 USPATFULL on STN
2002:294310 Compositions and methods of using capsid protein from Flaviviruses and Pestiviruses.

Weiner, David B., Merion Station, PA, UNITED STATES
Yang, Joo-Sung, Philadelphia, PA, UNITED STATES
US 2002164349 A1 20021107
APPLICATION: US 2001-971980 A1 20011004 (9)
PRIORITY: US 2000-237885P 20001004 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides methods of inducing cell death with Flavivirus or Pestivirus capsid protein, such as **West Nile virus** (WNV) capsid protein, and functional fragments thereof. The invention also provides methods of treating patients suffering from diseases characterized by hyperproliferating cells by administering pharmaceutical compositions comprising WNV or other virus including Flavivirus or Pestivirus capsid or other protein or a nucleic acid molecule encoding the same. Methods of identifying compounds which have anti-viral and/or anti-WNV and/or anti-Flavivirus and/or anti-Pestivirus capsid or other protein activity are disclosed. The invention also provides vaccine compositions comprising capsid or other proteins, or fragments thereof, or nucleic acids encoding same, from WNV or other virus including Flavivirus or Pestivirus and a pharmaceutically acceptable carrier. The invention also provides diagnostic methods and kits for identifying individuals exposed to WNV or other viruses including Flavivirus or Pestivirus.

CLM What is claimed is:

1. A method of inducing cell death comprising the step of contacting a cell with an amount of isolated Flavivirus or Pestivirus capsid protein, or a functional fragment thereof, effective to induce cell death; or introducing into said cell a nucleic acid molecule comprising a nucleotide sequence encoding a Flavivirus or Pestivirus capsid protein, or a functional fragment thereof, said nucleic acid being free from an entire Flavivirus or Pestivirus virus genome, wherein said nucleotide sequence is expressed in said cell at a level effective to induce cell death.

2. The method of claim 1, wherein the isolated capsid protein, or functional fragment thereof, or the nucleic acid molecule is from a virus selected from the Japanese encephalitis virus group subgenus.

3. The method of claim 1, wherein the isolated capsid protein, or functional fragment thereof, or the nucleic acid molecule is from **West Nile virus** (WNV).

4. The method of claim 3, wherein the functional fragment comprises SEQ ID NO:8.

5. The method of claim 3, wherein the nucleic acid molecule encodes SEQ ID NO:8.

6. The method of claim 1, wherein the cell is a tumor cell.

7. The method of claim 1, wherein the cell is contacted with the Flavivirus or Pestivirus capsid protein, or a functional fragment thereof.

8. The method of claim 1, wherein the nucleic acid molecule is introduced into said cell.

9. A method of identifying compounds that inhibit Flavivirus or Pestivirus capsid protein, or a functional fragment thereof, from inducing apoptosis in cells comprising the steps of a) contacting the cells, in the presence of a test compound, with an amount of Flavivirus or Pestivirus capsid protein, or a functional fragment thereof, sufficient to induce a detectable level of apoptosis in the cells; and b) comparing the level of apoptosis detected in step (a) with the level of apoptosis that occurs when cells are contacted with Flavivirus or Pestivirus capsid protein, or a functional fragment thereof, in the absence of said test compound.

10. The method of claim 9, wherein the cells are contacted with Flavivirus or Pestivirus capsid protein.

11. The method of claim 9, wherein the cells are contacted with a functional fragment of Flavivirus or Pestivirus capsid protein.

12. The method of claim 11, wherein the functional fragment comprises SEQ ID NO:8.

13. The method of claim 9, wherein the cells are selected from the group consisting of Hela cells, RD cells, and 293 cells.

14. The method of claim 9, wherein the detecting step is an assay that detects a marker of apoptosis.

15. The method of claim 14, wherein the marker is phosphatidylserine (PS) or free 3'-hydroxy DNA termini.

16. The method of claim 15, wherein the assay is TUNEL analysis or annexin V flow cytometry.

17. A kit for performing the method of claim 9 comprising a) a container comprising Flavivirus or Pestivirus capsid protein, or functional fragment thereof; and b) at least one additional component selected from the group consisting of: instructions, positive controls, negative controls, photos depicting data, and figures depicting data.

18. An injectable pharmaceutical composition comprising a) a Flavivirus or Pestivirus capsid protein, or a functional fragment thereof, or a nucleic acid molecule that comprises a nucleotide sequence that encodes a Flavivirus or Pestivirus capsid protein or a functional fragment thereof; and b) a pharmaceutically acceptable carrier.

19. The injectable pharmaceutical composition of claim 18 comprising a) a nucleic acid molecule that comprises a nucleotide sequence that encodes a Flavivirus or Pestivirus capsid protein or a functional fragment thereof; and b) a pharmaceutically acceptable carrier.

20. The injectable pharmaceutical composition of claim 18 comprising a) a Flavivirus or Pestivirus capsid protein, or a functional fragment thereof; and b) a pharmaceutically acceptable carrier.

21. The injectable pharmaceutical composition of claim 18 comprising a) a WNV capsid protein, or a functional fragment thereof; and b) a pharmaceutically acceptable carrier.

22. A method of treating an individual diagnosed with or suspected of suffering from a disease characterized by hyperproliferating cells which comprises the step of administering to said individual an effective amount of the injectable pharmaceutical composition of claim 18.

23. A method of treating an individual diagnosed with or suspected of suffering from a disease characterized by hyperproliferating cells which comprises the step of administering to said individual an effective amount of the injectable pharmaceutical composition of claim 19.

24. A method of treating an individual diagnosed with or suspected of suffering from a disease characterized by hyperproliferating cells which comprises the step of administering to said individual an effective amount of the injectable pharmaceutical composition of claim 20.

25. A method of treating an individual diagnosed with or suspected of suffering from a disease characterized by undesirable cells comprising eliminating the undesirable cells by administering to said individual an effective amount of the injectable pharmaceutical composition of claim 18.

26. The method of claim 24, wherein the capsid protein, or functional fragment thereof, is WNV capsid protein, or functional fragment thereof.

27. The method of claim 22, wherein the disease is cancer.

28. The method of claim 22, wherein the administration step is accomplished by intra-tumoral injection of the injectable pharmaceutical composition.

29. A method of identifying an individual exposed to Flavivirus or Pestivirus comprising the steps of: a) contacting antibodies specific for Flavivirus or Pestivirus capsid protein with a sample from the individual; and b) detecting whether said antibodies are bound to Flavivirus or Pestivirus capsid protein from the sample, wherein detection of binding of the antibodies to Flavivirus or Pestivirus capsid protein is indicative of exposure of the individual to Flavivirus or Pestivirus.

30. The method of claim 24, wherein the capsid protein is WNV capsid protein.

31. A kit for identifying individuals exposed to a Flavivirus or Pestivirus comprising a) a first container comprising antibodies specific for a Flavivirus or Pestivirus capsid protein; and b) a second

container comprising Flavivirus or Pestivirus capsid protein, or a fragment thereof.

32. The kit of claim 31, wherein the first container comprises antibodies specific for WNV capsid protein and the second container comprises WNV capsid protein, or a fragment thereof.

33. A method of identifying an individual exposed to a Flavivirus or Pestivirus comprising the steps of: a) contacting a sample with Flavivirus or Pestivirus capsid protein; and b) detecting whether said Flavivirus or Pestivirus capsid protein is bound to antibodies in said sample, wherein detection of binding of Flavivirus or Pestivirus capsid protein is indicative of exposure of the individual to Flavivirus or Pestivirus.

34. The method of claim 33, wherein the virus is WNV and the capsid protein is WNV capsid protein.

35. A kit for identifying individuals exposed to a Flavivirus or Pestivirus comprising a) a first container comprising Flavivirus or Pestivirus capsid protein; and b) a second container which contains antibodies which specifically bind to Flavivirus or Pestivirus capsid protein.

36. The kit of claim 35, wherein the capsid protein is WNV capsid protein.

37. A vaccine composition comprising a) an immunologically effective amount of Flavivirus or Pestivirus capsid protein, or an immunogenic fragment thereof; and b) a pharmaceutically acceptable carrier.

38. The vaccine of claim 37, wherein the Flavivirus or Pestivirus capsid protein, or immunogenic fragment thereof, is WNV capsid protein, or immunogenic fragment thereof.

39. A vaccine composition comprising a) nucleic acid encoding Flavivirus or Pestivirus capsid protein, or an immunogenic fragment thereof, and b) a pharmaceutically acceptable carrier.

40. The vaccine of claim 39, wherein the nucleic acid encodes WNV capsid protein, or an immunogenic fragment thereof.

41. A method of treating an individual exposed to a Flavivirus or Pestivirus by administering a therapeutically effective amount of capsid protein, or an immunogenic fragment thereof, from a Flavivirus or Pestivirus, or a nucleic acid encoding capsid protein, or an immunogenic fragment thereof, from a Flavivirus or Pestivirus.

42. The method of claim 41, wherein the virus to which the individual is exposed is WNV, and wherein the capsid protein, or fragment thereof, or the nucleic acid encoding the capsid protein, or immunogenic fragment thereof, is from WNV.

43. A method of protecting an individual from Flavivirus or Pestivirus infection by administering a prophylactically effective amount of capsid protein, or an immunogenic fragment thereof, from a Flavivirus or Pestivirus, or a nucleic acid encoding capsid protein, or an immunogenic fragment thereof, from a Flavivirus or Pestivirus.

44. The method of claim 43, wherein the virus against which the individual is to be protected is WNV, and wherein the capsid protein, or fragment thereof, or the nucleic acid encoding the capsid protein, or immunogenic fragment thereof, is from WNV.

L14 ANSWER 30 OF 34 USPATFULL on STN
2002:272441 Compositions and methods for tissue specific gene regulation therapy.
Vile, Richard G., Rochester, MN, UNITED STATES
Harrington, Kevin, London, UNITED KINGDOM
Murphy, Steven, Sudbury, UNITED KINGDOM
Bateman, Andrew, London, UNITED KINGDOM
US 2002150556 A1 20021017
APPLICATION: US 2001-822634 A1 20010330 (9)
PRIORITY: US 2000-193977P 20000331 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to a recombinant nucleic acid vector comprising a first expression cassette comprising a first promoter operably linked to

a nucleic acid sequence encoding a syncytium-inducing polypeptide, wherein the first expression cassette is flanked on either side by a site recognized by a recombinase. The invention also includes a second expression cassette comprising a tissue-specific promoter operably linked to a nucleic acid sequence encoding a recombinase. The invention also includes cells and compositions including these expression cassettes and methods of reducing tumor volume by expression of these expression cassettes.

CLM What is claimed is:

1. A recombinant nucleic acid vector comprising a first expression cassette comprising a first promoter operably linked to a nucleic acid sequence encoding a syncytium-inducing polypeptide, wherein said first expression cassette is flanked on either side by a site recognized by a recombinase.

2. The recombinant nucleic acid vector of claim 1, further comprising a second expression cassette comprising a tissue-specific promoter operably linked to a nucleic acid sequence encoding said recombinase.

3. The recombinant nucleic acid vector of claim 1, wherein said first promoter is active in malignant cells, and wherein said tissue specific promoter is active in non-malignant cells of the same lineage as said malignant cells but is substantially inactive in said malignant cells.

4. The recombinant nucleic acid vector of claim 1, wherein said recombinase is selected from the group consisting of Cre recombinase, FLP recombinase, Gin recombinase, Pin recombinase, and lambda phage Integrase, and said site is susceptible to cleavage with said recombinase.

5. The recombinant nucleic acid vector of claim 1, wherein said first promoter is a tumor-specific promoter.

6. The recombinant nucleic acid vector of claim 5, wherein said tumor specific promoter is selected from the group consisting of a carcinoembryonic antigen promoter, an alphafetoprotein promoter, a tyrosinase promoter, an Erb-B2 promoter and a myelin basic protein promoter.

7. The recombinant nucleic acid vector of claim 1, wherein said sequence which encodes a syncytium-inducing polypeptide encodes an FMG.

8. The recombinant nucleic acid vector of claim 7, wherein said FMG is a viral FMG.

9. The recombinant nucleic acid vector of claim 8, wherein said viral FMG is selected from the group consisting of type G membrane glycoprotein of rabies virus, type G membrane glycoprotein of Mokola virus, type G membrane glycoprotein of vesicular stomatitis virus, type G membrane glycoprotein of Togaviruses, murine hepatitis virus JHM surface projection protein, porcine respiratory coronavirus spike glycoprotein, porcine respiratory coronavirus membrane glycoprotein, avian infectious bronchitis spike glycoprotein and its precursor, bovine enteric coronavirus spike protein, paramyxovirus SV5 F protein, Measles virus F protein, canine distemper virus F protein, Newcastle disease virus F protein, human parainfluenza virus 3 F protein, simian virus 41 F protein, Sendai virus F protein, human respiratory syncytial virus F protein, Measles virus hemagglutinin, simian virus 41 hemagglutinin neuraminidase proteins, human parainfluenza virus type 3 hemagglutinin neuraminidase, Newcastle disease virus hemagglutinin neuraminidase, human herpesvirus 1 gH, simian varicella virus gH, human herpesvirus gB proteins, bovine herpesvirus gB proteins, cercopithecine herpesvirus gB proteins, Friend murine leukemia virus envelope glycoprotein, Mason Pfizer monkey virus envelope glycoprotein, HIV envelpoe glycoprotein, influenza virus hemaglutinin, poxvirus membrane glycoproteins, mumps virus hemaglutinin neuraminidase, mumps virus glycoproteins F1 and F2, **West Nile virus** membrane glycoprotein, herpes simplex virus membrane glycoprotein, Russian Far East encephalitis virus membrane glycoprotein, Venezuelan equine encephalitis virus membrane glycoprotein and varicella virus membrane glycoprotein.

10. The recombinant nucleic acid vector of claim 1, wherein said vector is a retroviral vector.

11. A cell comprising a vector of claim 1.

12. A recombinant expression cassette system comprising a first expression cassette comprising a first promoter operably linked to a nucleic acid sequence encoding a syncytium-inducing polypeptide, wherein

said first expression cassette is flanked on either side by a site recognized by a recombinase; and a second expression cassette comprising a tissue-specific promoter operably linked to a nucleic acid sequence encoding said recombinase.

13. The expression cassette system of claim 12, wherein said first and said second expression cassettes are encoded on a single vector nucleic acid.

14. The expression cassette system of claim 12, wherein said first and said second expression cassettes are encoded on separate nucleic acid vectors.

15. The expression cassette system of claim 12, wherein said first promoter is active in malignant cells, and wherein said tissue specific promoter is active in non-malignant cells of the same lineage as said malignant cells but is substantially inactive in said malignant cells.

16. The expression cassette system of claim 12, wherein said recombinase is selected from the group consisting of Cre recombinase, FLP recombinase, Gin recombinase, Pin recombinase, and lambda phage Integrase, and said site is susceptible to cleavage with said recombinase.

17. The expression cassette system of claim 12, wherein said first promoter is a tumor specific promoter.

18. The expression cassette system of claim 17, wherein said tumor specific promoter is selected from the group consisting of a carcinoembryonic antigen promoter, an alphafetoprotein promoter, a tyrosinase promoter, an Erb-B2 promoter and a myelin basic protein promoter.

19. The expression cassette system of claim 12, wherein said sequence which encodes a syncytium-inducing polypeptide encodes an FMG.

20. The expression cassette system of claim 19, wherein said FMG is a viral FMG.

21. The expression cassette system of claim 20 wherein said viral FMG is selected from the group consisting of type G membrane glycoprotein of rabies virus, type G membrane glycoprotein of Mokola virus, type G membrane glycoprotein of vesicular stomatitis virus, type G membrane glycoprotein of Togaviruses, murine hepatitis virus JHM surface projection protein, porcine respiratory coronavirus spike glycoprotein, porcine respiratory coronavirus membrane glycoprotein, avian infectious bronchitis spike glycoprotein and its precursor, bovine enteric coronavirus spike protein, paramyxovirus SV5 F protein, Measles virus F protein, canine distemper virus F protein, Newcastle disease virus F protein, human parainfluenza virus 3 F protein, simian virus 41 F protein, Sendai virus F protein, human respiratory syncytial virus F protein, Measles virus hemagglutinin, simian virus 41 hemagglutinin neuraminidase proteins, human parainfluenza virus type 3 hemagglutinin neuraminidase, Newcastle disease virus hemagglutinin neuraminidase, human herpesvirus 1 gH, simian varicella virus gH, human herpesvirus gB proteins, bovine herpesvirus gB proteins, cercopithecine herpesvirus gB proteins, Friend murine leukemia virus envelope glycoprotein, Mason Pfizer monkey virus envelope glycoprotein, HIV envelope glycoprotein, influenza virus hemaglutinin, poxvirus membrane glycoproteins, mumps virus hemaglutinin neuraminidase, mumps virus glycoproteins F1 and F2, **West Nile virus** membrane glycoprotein, herpes simplex virus membrane glycoprotein, Russian Far East encephalitis virus membrane glycoprotein, Venezuelan equine encephalitis virus membrane glycoprotein and varicella virus membrane glycoprotein.

22. The expression cassette system of any one of claims 12, wherein said expression cassette system is encoded one or more retroviral vectors.

23. A cell comprising the expression cassette system of claims 12.

24. A therapeutic composition comprising a cell of any one of claims 1 or 12 in admixture with a physiologically acceptable carrier.

25. A method of reducing tumor size, said method comprising the step of: (a) permitting expression in an individual in need of treatment for a disease caused by malignant cells of a first expression cassette comprising a tumor specific promoter operably linked to a nucleic acid sequence encoding a syncytium-inducing polypeptide, wherein said first expression cassette is flanked on either side by a site recognized by a

recombinase; and (b) a second expression cassette comprising a tissue-specific promoter operably linked to a nucleic acid sequence encoding said recombinase, wherein said tumor-specific promoter is active in said malignant cells, and said tissue specific promoter is active in non-malignant cells of the same lineage as the malignant cells, but substantially inactive in said malignant cells, wherein said expression results in a reduction in tumor size.

26. The method of claim 25, wherein said step of permitting expression comprises the step of administering first and second expression cassettes to an individual in need of treatment for a disease caused by malignant cells.

27. The method of claim 25, wherein said recombinase is cre recombinase and said site recognized by a recombinase is a loxp site.

28. The method of claim 25, wherein said tumor-specific promoter is selected from the group consisting of a carcino embryonic antigen promoter, an alphafetoprotein promoter, a tyrosinase promoter, an Erb-B2 promoter and a myelin basic protein promoter.

29. The method of claim 28, wherein said tumor specific promoter is the carcinoembryonic antigen promoter.

30. The method of claim 25, wherein said sequence which encodes a syncytium-inducing polypeptide encodes an FMG.

31. The method of claim 30, wherein FMG is a viral FMG.

32. The method of claim 31, wherein said viral FMG is selected from the group consisting of type G membrane glycoprotein of rabies virus, type G membrane glycoprotein of Mokola virus, type G membrane glycoprotein of vesicular stomatitis virus, type G membrane glycoprotein of Togaviruses, murine hepatitis virus JHM surface projection protein, porcine respiratory coronavirus spike glycoprotein, porcine respiratory coronavirus membrane glycoprotein, avian infectious bronchitis spike glycoprotein and its precursor, bovine enteric coronavirus spike protein, paramyxovirus SV5 F protein, Measles virus F protein, canine distemper virus F protein, Newcastle disease virus F protein, human parainfluenza virus 3 F protein, simian virus 41 F protein, Sendai virus F protein, human respiratory syncytial virus F protein, Measles virus hemagglutinin, simian virus 41 hemagglutinin neuraminidase proteins, human parainfluenza virus type 3 hemagglutinin neuraminidase, Newcastle disease virus hemagglutinin neuraminidase, human herpesvirus 1 gH, simian varicella virus gH, human herpesvirus gB proteins, bovine herpesvirus gB proteins, cercopithecine herpesvirus gB proteins, Friend murine leukemia virus envelope glycoprotein, Mason Pfizer monkey virus envelope glycoprotein, HIV envelpoe glycoprotein, influenza virus hemaglutinin, poxvirus membrane glycoproteins, mumps virus hemaglutinin neuraminidase, mumps virus glycoproteins F1 and F2, **West Nile virus** membrane glycoprotein, herpes simplex virus membrane glycoprotein, Russian Far East encephalitis virus membrane glycoprotein, Venezuelan equine encephalitis virus membrane glycoprotein and varicella virus membrane glycoprotein.

33. The method of claim 25, wherein said step of administering comprises administering one or more retroviral vectors comprising said first and second expression cassettes.

34. The method of claim 25, wherein said step of administering comprises administering a cell comprising said one or more recombinant nucleic acid vector.

35. An expression cassette system comprising: (a) a first expression cassette comprising an hypoxic response element (HRE) operably linked to a nucleic acid sequence encoding a syncytium-inducing polypeptide, wherein said nucleic acid sequence encoding a syncytium-inducing polypeptide is flanked on either side by a sequence recognized by a recombinase; (b) a second expression cassette comprising a tumor specific promoter operably linked to a nucleic acid sequence encoding a cytotoxic gene product; and (c) a third expression cassette comprising a tumor specific promoter operably linked to said nucleic acid sequence encoding said recombinase.

36. An expression cassette system comprising: (a) a first expression cassette comprising an hypoxic response element (HRE) operably linked to a nucleic acid sequence encoding a syncytium-inducing polypeptide, wherein said nucleic acid sequence encoding a syncytium-inducing polypeptide is flanked on either side by sequences recognized by a

recombinase; (b) a second expression cassette comprising a tumor specific promoter operably linked to a nucleic acid sequence encoding a cytokine; and (c) a third expression cassette comprising a tumor specific promoter operably linked to said nucleic acid sequence encoding said recombinase.

37. The expression cassette system of claim 35 or 36, wherein said vector is a retroviral vector.

38. The expression cassette system of claim 35 or 36, wherein said tumor specific promoter is selected from the group consiting of a carcinoembryonic antigen promoter, an alphafetoprotein promoter, a tyrosinase promoter, an Erb-B2 promoter and a myelin basic protein promoter.

39. The expression cassette system of claim 38, wherein said tumor specific promoter is a carcinoembryonic antigen promoter.

40. The expression cassette system of claim 35, wherein said cytotoxic gene product is selected from the group consisting of HSV thymidine kinase, cytosine deaminase, nitroreductase, and a viral FMG.

41. The expression cassette system of claim 36, wherein said cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-12, GM-CSF, IFN-γ and TNF-α.

42. A cell comprising an expression cassette system of claim 35 or 36.

43. The cell of claim 42, wherein said cell is a macrophage.

44. A method of reducing the size of a tumor in an individual said method comprising the step of permitting the expression in an individual of an expression cassette system comprising: (a) a first expression cassette comprising a nucleic acid sequence encoding a syncytium-inducing polypeptide, operably linked to an hypoxic response element (HRE), wherein said nucleic acid sequence encoding a syncytium-inducing polypeptide is flanked on either side by sequences recognized by a recombinase, (b) a second expression cassette comprising a nucleic acid sequence encoding a cytotoxic gene product, operably linked to a tumor specific promoter, and (c) a third expression cassette comprising a nucleic acid sequence encoding said recombinase, operably linked to said tumor specific promoter, wherein expression of said expression cassette system reduces the size of a tumor.

45. The method of claim 46, wherein said step of permitting expression comprises introducing said expression cassette system to a macrophage and introducing said macrophage to said individual.

46. A method of reducing the size of a tumor in an individual said method comprising the step of permitting the expression in an individual of an expression cassette system comprising: (a) a first expression cassette comprising a nucleic acid sequence encoding a syncytium-inducing polypeptide, operably linked to an hypoxic response element (HRE), wherein said nucleic acid sequence encoding a syncytium-inducing polypeptide is flanked on either side by sequences recognized by a recombinase, (b) a second expression cassette comprising a nucleic acid sequence encoding a cytokine, operably linked to a tumor specific promoter, and (c) a third expression cassette comprising a nucleic acid sequence encoding said recombinase, operably linked to said tumor specific promoter, wherein expression of said expression cassette system reduces the size of a tumor.

47. The method of claim 46, wherein said step of permitting expression comprises introducing said expression cassette system to a macrophage and introducing said macrophage to said individual.

48. A macrophage-tumor cell hybrid.

49. The macrophage-tumor cell hybrid of claim 48, wherein said hybrid comprises an expression cassette system of claims 35.

50. The macrophage-tumor cell hybrid of claim 48, wherein said hybrid comprises an expression cassette system of claims 36.

51. A cell-tumor cell hybrid, wherein said hybrid comprises a hypoxic transcription factor.

52. The cell-tumor cell hybrid of claim 51, wherein said hybrid an

expression cassette system of claim 35.

53. The cell-tumor cell hybrid of claim 51, wherein said hybrid an expression cassette system of claim 36.

L14 ANSWER 31 OF 34 USPATFULL on STN
2002:191503 Nucleic acid sensor molecules.
Usman, Nassim, Lafayette, CO, UNITED STATES
McSwiggen, James A., Boulder, CO, UNITED STATES
Zinnen, Shawn, Denver, CO, UNITED STATES
Seiwert, Scott, Lyons, CO, UNITED STATES
Haeberli, Peter, Berthoud, CO, UNITED STATES
Chowrira, Bharat, Broomfield, CO, UNITED STATES
Blatt, Lawrence, Boulder, CO, UNITED STATES
Vaish, Narendra K., Boulder, CO, UNITED STATES
US 2002102568 A1 20020801
APPLICATION: US 2001-877526 A1 20010608 (9)
PRIORITY: WO 2001-US7163 20010306
US 2000-187128P 20000306 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Nucleic acid sensor molecules and methods are disclosed for the detection and amplification of signaling agents using enzymatic nucleic acid constructs, including hammerhead enzymatic nucleic acid molecules, inozymes, G-cleaver enzymatic nucleic acid molecules, zinzymes, amberzymes and DNAzymes; kits for detection and amplification; use in diagnostics, nucleic acid circuits, nucleic acid computers, therapeutics, target validation, target discovery, drug optimization, SNP detection, SNP scoring, proteome scoring and other uses are disclosed.
CLM What is claimed is:
1. A nucleic acid sensor molecule comprising an enzymatic nucleic acid component and one or more sensor components, wherein, in response to an interaction of a single stranded RNA (ssRNA) having a single nucleotide polymorphism (SNP) with the nucleic acid sensor molecule in a system, the enzymatic nucleic acid component catalyzes a chemical reaction resulting in a detectable response.

2. A nucleic acid sensor molecule comprising an enzymatic nucleic acid component and one or more sensor components, wherein, in response to an interaction of a single stranded DNA (ssDNA) having a SNP with the nucleic acid sensor molecule in a system, the enzymatic nucleic acid component catalyzes a chemical reaction resulting in a detectable response.

3. A nucleic acid sensor molecule comprising an enzymatic nucleic acid sensor molecule in a system, and one or more sensor components, wherein, in response to an interaction of a single stranded RNA (ssRNA) with the nucleic acid sensor molecule in a system, the enzymatic nucleic acid component catalyzes a chemical reaction resulting in cleavage of a predetermined RNA molecule associated with a disease.

4. A nucleic acid sensor molecule comprising an enzymatic nucleic acid component and one or more sensor components, wherein, in response to an interaction of a single stranded DNA (ssDNA) with the nucleic acid sensor molecule in a system, the enzymatic nucleic acid component catalyzes a chemical reaction resulting in cleavage of a predetermined RNA molecule associated with a disease.

5. A nucleic acid sensor molecule comprising an enzymatic nucleic acid component and one or more sensor components, wherein, in response to an interaction of a peptide with the nucleic acid sensor molecule in a system, the enzymatic nucleic acid component catalyzes a chemical reaction resulting in cleavage of a peptide with the nucleic acid sensor molecule in a system, the enzymatic nucleic acid component catalyzes a chemical reaction resulting in cleavage of a predetermined RNA molecule associated with a disease.

6. A nucleic acid sensor molecule comprising an enzymatic nucleic acid component and one or more sensor components, wherein, in response to an interaction of a protein with the nucleic acid sensor molecule in a system, the enzymatic nucleic acid component catalyzes a chemical reaction resulting in cleavage of a predetermined RNA molecule associated with a disease.

7. A nucleic acid sensor molecule comprising an enzymatic nucleic acid component and one or more sensor components, wherein, in response to an interaction of a single stranded RNA (ssRNA) having a SNP with the

nucleic acid sensor molecule in a system, the enzymatic nucleic acid component catalyzes a chemical reaction resulting in ligation of a predetermined RNA molecule to another predetermined RNA molecule.

8. A nucleic acid sensor molecule comprising an enzymatic nucleic acid component and one or more sensor components, wherein, in response to an interaction of a single stranded DNA (ssDNA) having a SNP with the nucleic acid sensor molecule in a system, the enzymatic nucleic acid component catalyzes a chemical reaction resulting in ligation of a predetermined RNA molecule to another predetermined RNA molecule.

9. A method comprising: a. contacting the nucleic acid sensor molecule of claim 1 with a system comprising at least one ssRNA having a SNP under conditions suitable for the enzymatic nucleic acid component of the nucleic acid sensor molecule to catalyzes a chemical reaction resulting in a detectable response; and b. assaying for the chemical reaction resulting in a detectable response.

10. A method comprising: a. contacting the nucleic acid sensor molecule of claim 2 with a system comprising at least one ssDNA having a SNP under conditions suitable for the enzymatic nucleic acid component of the nucleic acid sensor molecule to catalyzes a chemical reaction resulting in a detectable response; and b. assaying for the chemical reaction resulting in a detectable response.

11. A method comprising contacting the nucleic acid sensor molecule of claim 3 with a system comprising at least one ssRNA under conditions suitable for the enzymatic nucleic acid component of the nucleic acid sensor molecule to cleave the predetermined RNA molecule.

12. A method comprising contacting the nucleic acid sensor molecule of claim 4 with a system comprising at least one ssDNA under conditions suitable for the enzymatic nucleic acid component of the nucleic acid sensor molecule to cleave the predetermined RNA molecule

13. A method comprising contacting the nucleic acid sensor molecule of claim 5 with a system comprising at least one peptide under conditions suitable for the enzymatic nucleic acid component of the nucleic acid sensor molecule to cleave the predetermined RNA molecule.

14. A method comprising contacting the nucleic acid sensor molecule of claim 6 with a system comprising at least one protein, under conditions suitable for the enzymatic nucleic acid component of the nucleic acid sensor molecule to cleave the predetermined RNA molecule.

15. A method comprising contacting the nucleic acid sensor molecule of claim 7 with a system comprising at least one ssRNA having a SNP under conditions suitable for the enzymatic nucleic acid component of the nucleic acid sensor molecule to ligate a predetermined RNA molecule to another predetermined RNA molecule.

16. A method comprising contacting the nucleic acid sensor molecule of claim 8 with a system comprising at least one ssDNA having a SNP under conditions suitable for the enzymatic nucleic acid component of the nucleic acid sensor molecule to ligate a predetermined RNA molecule to another predetermined RNA molecule.

17. The nucleic acid sensor molecule of any of claim 1 or claim 2, wherein said chemical reaction is cleavage of a phosphodiester internucleotide linkage.

18. The nucleic acid sensor molecule of any of claim 1 or claim 2, wherein said chemical reaction is ligation of a predetermined nucleic acid molecule to the nucleic acid sensor molecule.

19. The nucleic acid sensor molecule of claim 1 or claim 2, wherein said chemical reaction is ligation of a predetermined nucleic acid molecule to another predetermined nucleic acid molecule.

20. The nucleic acid sensor molecule of claim 1 or claim 2, wherein said chemical reaction is isomerization.

21. The nucleic acid sensor molecule of claim 1 or claim 2, wherein said chemical reaction is phosphorylation of a peptide or protein.

22. The nucleic acid sensor molecule of claim 1 or claim 2, wherein said chemical reaction is dephosphorylation of a peptide or protein.

23. The nucleic acid sensor molecule of claim 1 or claim 2, wherein said

chemical reaction is RNA polymerase activity.

24. The nucleic acid molecule of claim 1 or claim 2, wherein said detectable response is an increase or decrease in fluorescence.

25. The nucleic acid molecule of claim 1 or claim 2, wherein said detectable response is an increase or decrease in enzymatic activity.

26. The nucleic acid molecule of claim 1 or claim 2, wherein said detectable response is an increase or decrease in the production of a precipitate.

27. The nucleic acid molecule of claim 1 or claim 2, wherein said detectable response is an increase or decrease in chemoluminescence.

28. The nucleic acid molecule of claim 1 or claim 2, wherein said detectable response is an increase or decrease in radioactive emission.

29. A kit comprising the nucleic acid sensor molecule of claim 1.

30. A kit comprising the nucleic acid sensor molecule of claim 2.

31. A kit comprising the nucleic acid sensor molecule of claim 3.

32. A kit comprising the nucleic acid sensor molecule of claim 4.

33. A kit comprising the nucleic acid sensor molecule of claim 5.

34. A kit comprising the nucleic acid sensor molecule of claim 6.

35. A kit comprising the nucleic acid sensor molecule of claim 7.

36. A kit comprising the nucleic acid sensor molecule of claim 8.

37. The nucleic acid sensor molecule of claim 5, wherein said peptide is an Hepatitis C Virus (HCV) peptide.

38. The nucleic acid sensor molecule of claim 6, wherein said protein is an HCV protein.

39. The nucleic acid sensor molecule of claim 5 or claim 6, wherein said sensor component is a sequence derived from the HCV 5'-UTR.

40. The nucleic acid sensor molecule of claim 39, wherein said sequence derived from the HCV 5'-UTR is structural domain IIIa.

41. The nucleic acid sensor molecule of claim 39, wherein said sequence derived from the HCV 5'-UTR is structural domain IIIb.

42. The nucleic acid sensor molecule of claim 39, wherein said sequence derived from the HCV 5'-UTR is structural domain IIIc.

43. The nucleic acid sensor molecule of claim 39, wherein said sequence derived from the HCV 5'-UTR is structural domain IIId.

44. The nucleic acid sensor molecule of claim 39, wherein said sequence derived from the HCV 5'-UTR is structural domain IIIe.

45. The nucleic acid sensor molecule of claim 39, wherein said sequence derived from the HCV 5'-UTR is structural domain IIIf.

46. The nucleic acid sensor molecule of claim 39, wherein said sequence derived from the HCV 5'-UTR is structural domain I.

47. The nucleic acid sensor molecule of claim 39, wherein said sequence derived from the HCV 5'-UTR is structural domain II.

48. The nucleic acid sensor molecule of claim 39, wherein said sequence derived from the HCV 5'-UTR is structural domain IV.

49. The nucleic acid sensor molecule of claim 5, wherein said peptide is a viral peptide.

50. The nucleic acid sensor molecule of claim 49, wherein said viral peptide is derived from HCV, Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV), Human Papilloma Virus (HPV), Human T-cell Lyphotrophic Virus Type 1 (HTLV-1), Cytomegalovirus (CMV), Herpes Simplex Virus (HSV), respiratory syncytial virus (RSV), Rhinovirus, **West Nile Virus** (WNV), Hantavirus, Ebola virus, or Encephalovirus.

51. The nucleic acid sensor molecule of claim 6, wherein said protein is a viral protein.

52. The nucleic acid sensor molecule of claim 51, wherein said viral protein is derived from HCV, HBV, HIV, HPV, HTLV-1, CMV, HSV, RSV, Rhinovirus, WNV, Hantavirus, Ebola virus, or Encephalovirus.

53. The nucleic acid sensor molecule of claim 37 or claim 38, wherein said predetermined RNA is associated with HCV infection.

54. The nucleic acid sensor molecule of claim 39, wherein said predetermined RNA is associated with HCV infection.

L14 ANSWER 32 OF 34 USPATFULL on STN
2002:119310 Methods of preventing or treating **West Nile virus** and other infection.
Rahal, James J., New York, NY, UNITED STATES
US 2002061290 A1 20020523
APPLICATION: US 2001-935966 A1 20010823 (9)
PRIORITY: US 2000-227422P 20000823 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides methods of preventing or treating **West Nile virus** as well as infections caused by other viruses of the Flaviviridae family in animals comprising administering to the animal an effective amount of ribavirin and/or interferon alpha-2b.

CLM What is claimed is:

1. A method of preventing or treating **West Nile virus** in a human comprising administering to the human an effective amount of ribavirin.

2. A method in accordance with claim 1, wherein the ribavirin is administered orally.

3. A method in accordance with claim 2, wherein the ribavirin is administered in an amount from about 300 mg to about 3600 mg/day.

4. A method in accordance with claim 2, wherein the ribavirin is administered in an amount of 1200 mg as an initial dose, then 600 mg every 6 hours.

5. A method of preventing or treating **West Nile virus** in an animal suffering therefrom comprising administering to the animal an effective amount of interferon alpha-2b.

6. A method in accordance with claim 5, wherein the animal is a human.

7. A method in accordance with claim 6, wherein the interferon alpha-2b is administered parenterally to the human.

8. A method in accordance with claim 7, wherein the interferon alpha-2b is administered in an amount from about 1.5 million units to about 10 million units/day.

9. A method in accordance with claim 7, wherein the interferon alpha-2b is administered in an amount of 3 million units as an initial dose, then 3 million units every 12 to 24 hours.

10. A method of treating or preventing **West Nile virus** in an animal suffering therefrom comprising administering to the animal an effective amount of ribavirin and interferon alpha-2b.

11. A method in accordance with claim 10, wherein the animal is a human.

12. A method in accordance with claim 11, wherein the ribavirin is administered orally and the interferon alpha-2b is administered parenterally to the human.

13. A method in accordance with claim 12, wherein the ribavirin is administered to the human in an amount from about 300 mg to about 3600 mg/day and the interferon alpha-2b is administered in an amount from about 1.5 million units to about 10 million units/day.

14. A method in accordance with claim 13, wherein the ribavirin is administered to the human in an amount of 1200 mg as an initial dose, then 600 mg every 6 hours.

15. A method in accordance with claim 13, wherein the interferon

alpha-2b is administered to the human in an amount of 3 million units as an initial dose, then 3 million units every 12 to 24 hours.

16. A method of preventing or treating an animal with an infection, comprising: administering to the animal an effective amount of ribavirin, interferon alpha-2b or combinations thereof, wherein the infection causes an encephalitis selected from the group consisting of St. Louis, Japanese, and Murray Valley.

L14 ANSWER 33 OF 34 USPATFULL on STN

2001:185333 Inhibition and treatment of Hepatitis B virus and Flavivirus by Helioxanthin and its analogs.

Cheng, Yung-Chi, Woodbridge, CT, United States
Chou, Chen-Kung, Taipei, Taiwan, Province of China
Fu, Lei, Hamilton, Canada
Kuo, Yueh-Hsiung, Taipei, Taiwan, Province of China
Yeh, Sheau-Farn, Taipei, Taiwan, Province of China
Zhu, Juliang, Hamden, CT, United States
Zhu, Yonglian, New Haven, CT, United States
Yale University, New Haven, CT, United States (U.S. corporation)N. Y. Mu, N.T.U., V.G.H, Taipei, Taiwan, Province of China (non-U.S. corporation)

US 6306899 B1 20011023
APPLICATION: US 1999-379050 19990823 (9)
PRIORITY: US 1998-98025P 19980825 (60)
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to anti-viral drugs such as Helioxanthin and its analogs. The present compounds may be used alone or in combination with other drugs for the treatment of Hepatitis B virus (HBV), Hepatitis C virus (HCV), Yellow Fever, Dengue Virus, Japanese Encephalitis, **West Nile virus** and related flaviviruses. In addition, compounds according to the present invention can be used to prevent hepatoma secondary to virus infection as well as other infections or disease states which are secondary to the virus infection.

CLM What is claimed is:

1. A pure compound according to the structure: ##STR6## where A is H, OH, OR or forms a 1,3 dioxolane group with B such that A and B are O and are bridged together by a $--CH_2$ -- group; C is H, OH, OR or forms a 1,3 dioxolane group with B such that B and C are O and are bridged together by a $--CH_2$ -- group; B is OH, OR or forms a 1,3 dioxolane group with either A or C; R is a C_1 to C_3 alkyl group, benzyl group or a C_1 to C_{20} acyl group; D and E are the same or different and are selected from CH_3, CH_2 OH, CH_2 OR, CHO, COOH or a pharmaceutically acceptable salt thereof, CH_2 $COOR^1$ or a keto group or $--CH_2$ -- group, with the proviso that when D or E is a keto group, the other of D or E is a keto group or a methylene group and D and E are linked together by an --O-- group to form a five-membered lactone ring or a dicarboxylic acid anhydride ring and with the further proviso that when B and A form a 1,3 dioxolane ring and when J and K form a 1,3, dioxolane ring, D and E do not form a lactone group where D is a keto group and E is a methylene group; R^1 is a C_1 to C_3 alkyl group; F and G are H or Br; I is H, OH, OR or Br and J and K are the same or different and are selected from CH_3, CH_2 OH, CH_2 OR, CHO, COOH or a pharmaceutically acceptable salt thereof or together form a 1,3 dioxolane ring such that J and K are O and are bridged by a $--CH_2$ -- group.

2. The compound according to claim 1, structure I, wherein G and F are Br.

3. The compound according to claim 1, structure I, wherein C, F and I are H, G is Br, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group and D and E are each CH_2 OH groups.

4. The compound according to claim 1, structure I, wherein C, F, G and I are H, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group, D is a CH_2 OH group and E is a CH_3 group.

5. The compound according to claim 1, structure I, wherein C, F, G and I are H, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group, D is a COO- Na+ group and E is a CH_2 OH group.

6. The compound according to claim 1, structure I, wherein A, F, G and I are H, B and C together form a 1,3 dioxolane group, D is a methylene

group, E is a keto group and D and E are linked together through an --O-- to form a five-membered lactone ring and J and K together form a 1,3 dioxolane group.

7. A method of treating a patient for a viral infection selected from the group consisting of Hepatitis B virus, Hepatitis C virus, Yellow Fever, Dengue Virus, Japanese Encephalitis and **West Nile virus** comprising administering to said patient an effective amount of a compound according to the structure: ##STR7## where A is H, OH, OR or forms a 1,3 dioxolane group with B such that A and B are O and are bridged together by a $--CH_2$ -- group; C is H, OH, OR or forms a 1,3 dioxolane group with B such that B and C are O and are bridged together by a $--CH_2$ -- group; B is OH, OR or forms a 1,3 dioxolane group with either A or C; R is a C_1 to C_3 alkyl group or a C_1 to C_{20} acyl group; D and E are the same or different and are selected from CH_3, CH_2 OH, CH_2 OR, CHO, COOH or a pharmaceutically acceptable salt thereof, CH_2 $COOR^1$ or a keto group or $--CH_2$ -- group, with the proviso that when D or E is a keto group, the other of D or E is a keto group or a methylene group and D and E are linked together by an --O-- group to form a five-membered lactone ring or a dicarboxylic acid anhydride ring; R^1 is a C_1 to C_3 alkyl group; F and G are H or Br; I is H, OH, OR or Br and J and K are the same or different and are selected from CH_3, CH_2 OH, CH_2 OR, CHO, COOH, COO- Na+ or together form a 1,3 dioxolane ring such that J and K are O and are bridged by a $--CH_2$ -- group.

8. The method according to claim 7 wherein said virus is HBV or Yellow Fever virus and wherein said compound is according to structure I, wherein G and F are Br.

9. The method according to claim 7 wherein said virus is Hepatitis B virus, Hepatitis C virus or Yellow Fever virus and wherein said compound is according to structure I, wherein C, F, G and I are H, A and B form a 1,3 dioxolane ring, D is a keto group, E is a methylene group and D and E are linked together by an --O-- to form a five-membered lactone ring and J and K together form a 1,3 dioxolane ring.

10. The method according to claim 7, wherein said virus is Hepatitis B virus, Hepatitis C virus or Yellow Fever virus and wherein said compound is according to structure I, wherein C, F and I are H, G is Br, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group and D and E are each CH_2 OH groups.

11. The method according to claim 7, wherein said virus is Hepatitis B virus, Hepatitis C virus or Yellow Fever virus and wherein said compound is according to structure I, wherein C, F, G and I are H, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group, D is a CH_2 OH group and E is a CH_3 group.

12. The method according to claim 7, wherein said virus is Hepatitis B virus, Hepatitis C virus or Yellow Fever virus and wherein said compound is according to structure I, wherein C, F, G and I are H, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group, D is a COO- Na+ group and E is a CH_2 OH group.

13. The method according to claim 7, wherein said virus is Hepatitis B virus, Hepatitis C virus or Yellow Fever Virus and wherein said compound is according to structure I, wherein A, F, G and I are H, B and C together form a 1,3 dioxolane group, D is a methylene group, E is a keto group and D and E are linked together through an --O-- to form a five-membered lactone ring and J and K together form a 1,3 dioxolane group.

14. A pharmaceutical composition for use in treating a virus infection comprising an anti-viral effective amount of at least one compound according to the structure: ##STR8## where A is H, OR or forms a 1,3 dioxolane group with B such that A and B are O and are bridged together by a $--CH_2$ -- group; C is H, OH, OR or forms a 1,3 dioxolane group with B such that B and C are O and are bridged together by a $--CH_2$ -- group; B is OH, OR or forms a 1,3 dioxolane group with either A or C; R is a C_1 to C_3 alkyl group, a benzyl group or a C_1 to C_{20} acyl group; D and E are the same or different and are selected from CH_3, CH_2 OH, CH_2 OR, CHO, COOH or a pharmaceutically acceptable salt thereof, CH_2 $COOR^1$ or a keto group or $--CH_2$ -- group, with the proviso that when D or E is a keto group, the other of D or E is a keto group or a methylene group and D and E are

linked together by an --O-- group to form a five-membered lactone ring or a dicarboxylic acid anhydride ring; R^1 is a C_1 to C_3 alkyl group; F and G are H or Br; I is H, OH, OR or Br and J and K are the same or different and are selected from CH_3, CH_2 OH, CH_2 OR, CHO, COOH or a pharmaceutically acceptable salt thereof, or together form a 1,3 dioxolane ring such that J and K are O and are bridged by a --CH_2 -- (methylene) group, optionally in combination with a pharmaceutically acceptable excipient, carrier or additive.

15. The composition according to claim 14, wherein said compound is according to structure I, wherein G and F are Br.

16. The composition according to claim 14, wherein said compound is according to structure I, wherein C, F, G and I are H, A and B form a 1,3 dioxolane ring, D is a keto group, E is a methylene group and D and E are linked together by an --O-- to form a five-membered lactone ring and J and K together form a 1,3 dioxolane ring.

17. The composition according to claim 14, wherein said compound is according to structure I, wherein C, F and I are H, G is Br, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group and D and E are each CH_2 OH groups.

18. The composition according to claim 14, wherein said compound is according to structure I, wherein C, F, G and I are H, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group, D is a CH_2 OH group and E is a CH_3 group.

19. The composition according to claim 14, wherein said compound is according to structure I, wherein C, F, G and I are H, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group, D is a COO- Na+ group and E is a CH_2 OH group.

20. The composition according to claim 14, wherein said compound is according to structure I, wherein A, F, G and I are H, B and C together form a 1,3 dioxolane group, D is a methylene group, E is a keto group and D and E are linked together through an --O-- to form a five-membered lactone ring and J and K together form a 1,3 dioxolane group.

21. A method of preventing a virus infection in a patient, said virus being selected from the group consisting of Hepatitis B virus, Hepatitis C virus, Yellow Fever virus, Dengue Virus, Japanese Encephalitis virus and **West Nile virus** comprising administering to said patient in need thereof an effective amount of a compound according to the structure: ##STR9## where A is H, OR or forms a 1,3 dioxolane group with B such that A and B are O and are bridged together by a --CH_2 -- group; C is H, OH, OR or forms a 1,3 dioxolane group with B such that B and C are O and are bridged together by a --CH_2 -- group; B is OH, OR or forms a 1,3 dioxolane group with either A or C; R is a C_1 to C_3 alkyl group, a benzyl group or a C_1 to C_{20} acyl group D and E are the same or different and are selected from CH_3, CH_2 OH, CH_2 OR, CHO, COOH or a pharmaceutically acceptable salt thereof, CH_2 $COOR^1$ or a keto group or --CH_2 -- group, with the proviso that when D or E is a keto group, the other of D or E is a keto group or a methylene group and D and E are linked together by an --O-- group to form a five-membered lactone ring or a dicarboxylic acid anhydride ring; R^1 is a C_1 to C_3 alkyl group; F and G are H or Br; I is H, OH, OR or Br and J and K are the same or different and are selected from CH_3, CH_2 OH, CH_2 OR, CHO, COOH or a pharmaceutically acceptable salt thereof, or together form a 1,3 dioxolane ring such that J and K are O and are bridged by a --CH_2 -- group, optionally in combination with a pharmaceutically acceptable excipient, carrier or additive.

22. The method according to claim 21 wherein said virus is Hepatitis B virus, Hepatitis C virus or Yellow Fever virus and wherein said compound is according to structure I, wherein G and F are Br.

23. The method according to claim 21 wherein said virus is Hepatitis B virus, Hepatitis C virus or Yellow Fever virus and wherein said compound is according to structure I, wherein C, F, G and I are H, A and B form a 1,3 dioxolane ring, D is a keto group, E is a methylene group and D and E are linked together by an --O-- to form a five-membered lactone ring and J and K together form a 1,3 dioxolane ring.

24. The method according to claim 21, wherein said virus is Hepatitis B virus, Hepatitis C virus or Yellow Fever virus and wherein said compound is according to structure I, wherein C, F and I are H, G is Br, A and B

together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group and D and E are each CH_2 OH groups.

25. The method according to claim 21, wherein said virus is Hepatitis B virus, Hepatitis C virus or Yellow Fever virus and wherein said compound is according to structure I, wherein C, F, G and I are H, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group, D is a CH_2 OH group and E is a CH_3 group.

26. The method according to claim 21, wherein said virus is Hepatitis B virus, Hepatitis C virus or Yellow Fever virus and wherein said compound is according to structure I, wherein C, F, G and I are H, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group, D is a COO^- Na^+ group and E is a CH_2 OH group.

27. The method according to claim 21, wherein said virus is Hepatitis B virus, Hepatitis C virus or Yellow Fever Virus and wherein said compound is according to structure I, wherein A, F, G and I are H, B and C together form a 1,3 dioxolane group, D is a methylene group, E is a keto group and D and E are linked together through an --O-- to form a five-membered lactone ring and J and K together form a 1,3 dioxolane group.

28. A method of preventing a hepatoma secondary to a Hepatitis B or Hepatitis C virus infection in a patient, said method comprising administering to said patient in need thereof an effective amount of a compound according to the structure: ##STR10## where A is H, OR or forms a 1,3 dioxolane group with B such that A and B are O and are bridged together by a $--CH_2$ -- group; C is H, OH, OR or forms a 1,3 dioxolane group with B such that B and C are O and are bridged together by a $--CH_2$ -- group; B is OH, OR or forms a 1,3 dioxolane group with either A or C; R is a C_1 to C_3 alkyl group, a benzyl group or a C_1 to C_{20} acyl group D and E are the same or different and are selected from CH_3, CH_2 OH, CH_2 OR, CHO, COOH or a pharmaceutically acceptable salt thereof, CH_2 $COOR^1$ or a keto group or $--CH_2$ -- group, with the proviso that when D or E is a keto group, the other of D or E is a keto group or a methylene group and D and E are linked together by an --O-- group to form a five-membered lactone ring or a dicarboxylic acid anhydride ring; R^1 is a C_1 to C_3 alkyl group; F and G are H or Br; I is H, OH, OR or Br and J and K are the same or different and are selected from CH_3, CH_2 OH, CH_2 OR, CHO, COOH or a pharmaceutically acceptable salt thereof, or together form a 1,3 dioxolane ring such that J and K are O and are bridged by a $--CH_2$ -- group, optionally in combination with a pharmaceutically acceptable excipient, carrier or additive.

29. The method according to claim 28 wherein said compound is according to structure I, wherein G and F are Br.

30. The method according to claim 28 wherein said compound is according to structure I, wherein C, F, G and I are H, A and B form a 1,3 dioxolane ring, D is a keto group, E is a methylene group and D and E are linked together by an --O-- to form a five-membered lactone ring and J and K together form a 1,3 dioxolane ring.

31. The method according to claim 28, wherein said compound is according to structure I, wherein C, F and I are H, G is Br, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group and D and E are each CH_2 OH groups.

32. The method according to claim 28, wherein said compound is according to structure I, wherein C, F, G and I are H, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group, D is a CH_2 OH group and E is a CH_3 group.

33. The method according to claim 28, wherein said compound is according to structure I, wherein C, F, G and I are H, A and B together form a 1,3 dioxolane group, J and K together form a 1,3 dioxolane group, D is a COO^- Na^+ group and E is a CH_2 OH group.

34. The method according to claim 28, wherein said virus is Hepatitis B virus, Hepatitis C virus or Yellow Fever Virus and wherein said compound is according to structure I, wherein A, F, G and I are H, B and C together form a 1,3 dioxolane group, D is a methylene group, E is a keto group and D and E are linked together through an --O-- to form a five-membered lactone ring and J and K together form a 1,3 dioxolane

group.

35. The method according to claim 7 wherein said viral infection is a 3TC-resistant Hepatitis B virus infection.

36. The method according to claim 9 wherein said viral infection is a 3TC-resistant Hepatitis B virus infection.

37. A pharmaceutical composition comprising an anti-viral effective amount of at least one compound according to the structure: ##STR11## where C, G, F and I are H; A is O and B is O such that A and B are bridged together by a $--CH_2$ -- group to form a 1,3-dioxolane group; J is O and K is O such that J and K are bridged together by a $--CH_2$ -- group to form a 1,3-dioxolane group; D is COOH or a pharmaceutically acceptable salt thereof, or a keto group; and E is CH_2 OH when D is COOH or its pharmaceutically acceptable salt thereof, or a methylene group when D is a keto group such that D and E are linked together by an --O-- group to form a five-membered lactone ring, optionally, in combination with a pharmaceutically acceptable excipient carrier or additive.

38. The composition according to claim 37 where D is a keto group and E is a methylene group such that D and E are linked together by an --O-- group to form a five-membered lactone ring.

39. A method of treating a patient for a viral infection selected from the group consisting of Hepatitis B virus, Hepatitis C virus, Yellow Fever, Dengue Virus, Japanese Encephalitis, **West Nile virus** comprising administering to said patient an effective amount of a compound according to the structure: ##STR12## where C, G, F and I are H; A is O and B is O such that A and B are bridged together by a $--CH_2$ -- group to form a 1,3-dioxolane group; J is O and K is O such that J and K are bridged together by a $--CH_2$ -- group to form a 1,3-dioxolane group; D is COOH or a pharmaceutically acceptable salt thereof, or a keto group; and E is CH_2 OH when D is COOH or its pharmaceutically acceptable salt thereof, or a methylene group when D is a keto group such that D and E are linked together by an --O-- group to form a five-membered lactone ring, optionally, in combination with a pharmaceutically acceptable excipient carrier or additive.

40. The method according to claim 39 where D is a keto group and E is a methylene group such that D and E are linked together by an --O-- group to form a five-membered lactone ring.

41. The method according to claim 40 wherein said virus is Hepatitis C virus.

42. The method according to claim 40 wherein said virus is Hepatitis B virus.

43. The method according to claim 40 wherein said virus is 3TC resistant Hepatitis B virus.

44. A method of preventing a virus infection in a patient, said virus being selected from the group consisting of Hepatitis B virus, Hepatitis C virus, Yellow Fever virus, Dengue Virus, Japanese Encephalitis virus and **West Nile virus** comprising administering to said patient in need thereof an effective amount of a compound according to the structure: ##STR13## where C, G, F and I are H; A is O and B is O such that A and B are bridged together by a $--CH_2$ -- group to form a 1,3-dioxolane group; J is O and K is O such that J and K are bridged together by a $--CH_2$ -- group to form a 1,3-dioxolane group; D is COOH or a pharmaceutically acceptable salt thereof, or a keto group; and E is CH_2 OH when D is COOH or its pharmaceutically acceptable salt thereof, or a methylene group when D is a keto group such that D and E are linked together by an --O-- group to form a five-membered lactone ring, optionally, in combination with a pharmaceutically acceptable excipient carrier or additive.

45. The method according to claim 44 where D is a keto group and E is a methylene group such that D and E are linked together by an --O-- group to form a five-membered lactone ring.

46. The method according to claim 45 wherein said virus is Hepatitis C virus.

47. The method according to claim 45 wherein said virus is Hepatitis B virus.

48. The method according to claim 47 wherein said virus is 3TC resistant.

49. A method of preventing a hepatoma secondary to a Hepatitis B or Hepatitis C virus infection in a patient, said method comprising administering to said patient in need thereof an effective amount of a compound according to the structure: ##STR14## where C, G, F and I are H; A is O and B is O such that A and B are bridged together by a $--CH_2$ -- group to form a 1,3-dioxolane group; J is O and K is O such that J and K are bridged together by a $--CH_2$ -- group to form a 1,3-dioxolane group; D is COOH or a pharmaceutically acceptable salt thereof, or a keto group; and E is CH_2 OH when D is COOH or its pharmaceutically acceptable salt thereof, or a methylene group when D is a keto group such that D and E are linked together by an --O-- group to form a five-membered lactone ring, optionally, in combination with a pharmaceutically acceptable excipient carrier or additive.

50. The method according to claim 49 where D is a keto group and E is a methylene group such that D and E are linked together by an --O-- group to form a five-membered lactone ring.

51. The method according to claim 49 wherein said virus is Hepatitis C virus.

L14 ANSWER 34 OF 34 USPATFULL on STN
2001:182109 Induction of immunoglobulin class switching by inactivated viral vaccine.
Compans, Richard W., Atlanta, GA, United States
Sha, Zhiyi, Atlanta, GA, United States
US 2001031266 A1 20011018
APPLICATION: US 2000-733166 A1 20001208 (9)
PRIORITY: US 1999-169813P 19991208 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present disclosure provides methods and compositions for inducing an immune response to an antigen, especially in an immunogenic composition comprising sialic acid where the antigen comprises sialic acid and wherein the immunogenic composition further comprises a sialic acid binding component, e.g., an inactivated or attenuated paramyxovirus or orthomyxovirus such as an influenza virus comprising a sialic acid binding component, e.g., a neuraminidase. The compositions comprising sialic acid and a sialic acid binding component effectively induce a humoral immune response even in a human or animal which is deficient in CD4+ T cells, due to a disease such as ARC or AIDS, and there is also an immunoglobulin class switching even in the absence of CD4+ T cells.

CLM What is claimed:

1. A method for inducing an immune response in a human or animal wherein said human or animal has a deficiency in CD4+ T cells, said method comprising the step of administering to a human or animal deficient in T cells an immunogenic composition comprising a sialic acid binding component and at least one antigen of a target cell or target virus, whereby a humoral immune response specific for at least one antigen of the target cell or target virus is induced.

2. The method of claim 1 wherein the immune response is a humoral immune response.

3. The method of claim 1 wherein said sialic acid binding component is a hemagglutinin.

4. The method of claim 2 wherein said hemagglutinin is a viral hemagglutinin.

5. The method of claim 4 wherein said viral hemagglutinin is from an orthomyxovirus.

6. The method of claim 5 wherein said viral hemagglutinin is from influenza virus.

7. The method of claim 4 wherein said viral hemagglutinin is from a paramyxovirus.

8. The method of claim 4 wherein said viral hemagglutinin is comprised in an attenuated virus preparation.

9. The method of claim 4 wherein said viral hemagglutinin is comprised within an inactivated virus preparation.

10. The method of claim 8 wherein the virus preparation is inactivated with formalin or propiolactone.

11. The method of claim 4 wherein the at least one antigen of a target cell is from a bacterial pathogen cell.

12. The method of claim 11 wherein the bacterial pathogen cell has a sialic acid capsule and wherein said capsule in present in said immunogenic composition.

13. The method of claim 12 wherein said bacterial pathogen is Neisseria meningitidis.

14. The method of claim 12 wherein said bacterial pathogen is Eseherichia coli.

15. The method of claim 2 herein said target cell is a tumor cell.

16. The method of claim 2 wherein said target virus is an enveloped virus.

17. The method of claim 16 wherein said enveloped virus is simian immunodeficiency virus, human immunodeficiency virus, feline immunodeficiency virus, or bovine immunodeficiency virus, rabies virus, measles virus, vesicular stomatitis virus, flavivirus, alphavirus or herpes virus.

18. The method of claim 17 wherein said alphavirus is Sindbis virus, Semliki forest virus, Venezuelan equine encephalitis virus, eastern equine encephalitis virus, western equine encephalitis virus, Ross River virus, Mayaro virus, O'nyong-nyong virus or chikungunya virus.

19. The method of claim 17 wherein the flavivirus is Dengue virus, yellow fever virus, St. Louis encephalitis virus, Japanese encephalitis virus, Murray Valley encephalitis virus, **West Nile virus**, Rocio virus, tick-bome encephalitis virus, Omsk hemorrhagic fever virus, Kyasanur Forest disease virus, or Powassan virus.

20. The method of claim 9 wherein the immunogenic composition comprises an inactivated virus comprising a hemagglutinin or inactivated target cell or target virus and a carrier.

21. An immunogenic composition comprising a sialic acid binding component and an inactivated or attenuated target cell or an inactivated or attenuated target virus.

22. The immunogenic composition of claim 21 wherein said sialic acid binding component is a hemagglutinin of an orthomyxovirus or a paramyxovirus.

23. The immunogenic composition of claim 21 wherein said sialic acid binding component is comprised in an inactivated or attenuated preparation of an orthomyxovirus or paramyxovirus.

24. The immunogenic composition of claim 22 further comprising a virus like particle or an inactivated or attenuated sialic acid containing virus preparation.

25. The immunogenic composition of claim 24 wherein said virus preparation is an enveloped virus preparation.

26. The immunogenic composition of claim 25 wherein said is an inactivated tumor cell. virus preparation is a preparation of simian immunodeficiency virus, human immunodeficiency virus, feline immunodeficiency virus, or bovine immunodeficiency virus, rabies virus, measles virus, vesicular stomatitis virus, flavivirus, alphavirus or herpes virus.

27. The immunogenic composition of claim 26 wherein said alphavirus is Sindbis virus, Semliki forest virus, Venezuelan equine encephalitis virus, eastern equine encephalitis virus, western equine encephalitis virus, Ross River virus, Mayaro virus, O'nyong-nyong virus or chikungunya virus.

28. The immunogenic composition of claim 26 wherein the flavivirus is Dengue virus, yellow fever virus, St. Louis encephalitis virus, Japanese encephalitis virus, Murray Valley encephalitis virus, **West Nile virus**, Rocio virus, tick-borne encephalitis virus, Omsk hemorrhagic

fever virus, Kyasanur Forest disease virus, or Powassan virus.

29. The immunogenic composition of claim 21 wherein the target cell is a tumor cell.

30. The method of claim 21 wherein the at least one antigen of a target cell is from a bacterial pathogen cell.

31. The method of claim 30 wherein the bacterial pathogen cell has a sialic acid capsule and wherein said capsule in present in said immunogenic composition.

32. The method of claim 31 wherein said bacterial pathogen is Neisseria meningitidis.

33. The method of claim 30 wherein said bacterial pathogen is Escherichia coli.

34. An immunogenic composition comprising a sialic acid binding component and at least one antigen of a target cell or target virus.

35. The immunogenic composition of claim 34 wherein the sialic acid binding component is a hemagglutinin of an orthomyxovirus or a paramyxovirus.

36. The immunogenic composition of claim 35 wherein the composition comprises inactivated or attenuated orthomyxovirus or paramyxovirus.

37. The immunogenic composition of claim 34 wherein the at least one antigen of a target cell or target virus comprises sialic acid or polymerized sialic acid.

38. The immunogenic composition of claim 37 wherein the at least one antigen of a target cell or target virus is comprised within inactivated or attenuated target cell or inactivated or attenuated target virus or virus-like particles of a target virus.

39. The immunogenic composition of claim 38 wherein the target cell is Neisseria meningitidis or Escherichia coli.

40. The immunogenic composition of claim 38 wherein the target virus is simian immunodeficiency virus, human immunodeficiency virus, feline immunodeficiency virus, or bovine immunodeficiency virus, rabies virus, measles virus, vesicular stomatitis virus, flavivirus, alphavirus or herpes virus.

41. The immunogenic composition of claim 40 wherein said alphavirus is Sindbis virus, Semliki forest virus, Venezuelan equine encephalitis virus, eastern equine encephalitis virus, western equine encephalitis virus, Ross River virus, Mayaro virus, O'nyong-nyong virus or chikungunya virus.

42. The immunogenic composition of claim 40 wherein the flavivirus is Dengue virus, yellow fever virus, St. Louis encephalitis virus, Japanese encephalitis virus, Murray Valley encephalitis virus, **West Nile virus**, Rocio virus, tick-borne encephalitis virus, Omsk hemorrhagic fever virus, Kyasanur Forest disease virus, or Powassan virus.

43. The immunogenic composition of claim wherein the target cell is a tumor cell.

44. A method for inducing an immune response in a human or animal, said method comprising the steps of administering an immunogenic composition comprising a sialic acid binding compo component and at least one antigen of a target cell or target virus, whereby a humoral immune response specific for at least one antigen of the target cell or target virus is induced.

45. The method of claim 44 wherein the immune response is a humoral immune response.

46. The method of claim 45 wherein said sialic acid binding component is a hemagglutinin.

47. The method of claim 46 wherein said hemagglutinin is a viral hemagglutinin.

48. The method of claim 47 wherein said viral hemagglutinin is from an orthomyxovirus or a paramyxovirus.

49. The method of claim 48 wherein said viral hemagglutinin is from influenza virus.

50. The method of claim 47 wherein said viral hemagglutinin is comprised in an attenuated virus preparation.

51. The method of claim 47 wherein said viral hemagglutinin is comprised within an inactivated virus preparation.

52. The method of claim 51 wherein the virus preparation is inactivated with formalin or propiolactone.

53. The method of claim 46 wherein the at least one antigen of a target cell is from a bacterial pathogen cell.

54. The method of claim 53 wherein the bacterial pathogen cell has a sialic acid capsule and wherein said capsule in present in said immunogenic composition.

55. The method of claim 54 wherein said bacterial pathogen is Neisseria meningitidis or Escherichia coli.

56. The method of claim 46 wherein said target cell is a tumor cell.

57. The method of claim 56 wherein said target virus or is an enveloped virus.

58. The method of claim 57 wherein said enveloped virus is simian immunodeficiency virus, human immunodeficiency virus, feline immunodeficiency virus, or bovine immunodeficiency virus, rabies virus, measles virus, vesicular stomatitis virus, flavivirus, alphavirus or herpes virus.

59. The method of claim 58 wherein said alphavirus is Sindbis virus, Semliki forest virus, Venezuelan equine encephalitis virus, eastern equine encephalitis virus, western equine encephalitis virus, Ross River virus, Mayaro virus, O'nyong-nyong virus or chikungunya virus.

60. The method of claim 58 wherein the flavivirus is Dengue virus, yellow fever virus, St. Louis encephalitis virus, Japanese encephalitis virus, Murray Valley encephalitis virus, **West Nile virus**, Rocio virus, tick-borne encephalitis virus, Omsk hemorrhagic fever virus, Kyasanur Forest disease virus, or Powassan virus.

61. The method of claim 62 wherein the immunogenic composition comprises an inactivated virus comprising a hemagglutinin or inactivated target cell or target virus and a carrier.

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     (FILE 'HOME' ENTERED AT 15:35:18 ON 23 FEB 2004)

     FILE 'USPATFULL' ENTERED AT 15:35:57 ON 23 FEB 2004
L1           661 S YELLOW FEVER VIRUS
L2            59 S L1 AND (YELLOW FEVER VIRUS/CLM)
L3            17 S L2 AND (PRM AND E)
L4             4 S L3 AND (PRM/CLM)
L5            13 S L3 NOT L4
L6             7 S L3 AND E/CLM
L7           231 S ST. LOUIS ENCEPHALITIS VIRUS
L8            16 S L7 AND LOUIS/CLM
L9            13 S L8 NOT L5
L10           52 S POWASSAN VIRUS
L11            6 S L10 AND POWASSAN/CLM
L12            2 S L11 NOT L9
L13          264 S WEST NILE VIRUS
L14           34 S L13 AND (WEST NILE VIRUS/CLM)

=> file medline
COST IN U.S. DOLLARS                        SINCE FILE      TOTAL
                                              ENTRY    SESSION
FULL ESTIMATED COST                           245.64     245.85

FILE 'MEDLINE' ENTERED AT 16:30:55 ON 23 FEB 2004

 FILE LAST UPDATED: 21 FEB 2004 (20040221/UP).  FILE COVERS 1958 TO DATE.
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On December 14, 2003, the 2004 MeSH terms were loaded. See HELP RLOAD for details.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2004 vocabulary. See http://www.nlm.nih.gov/mesh/ and http:\\www.nih.gov/pubs/yechbull/nd03/nd03_mesh.html for a description on changes.

This file contains CAS Registry Numbers for easy and accurate substance identification.

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=> s powassan virus
            98 POWASSAN
        363928 VIRUS
L15         65 POWASSAN VIRUS
               (POWASSAN(W)VIRUS)

=> s l15 and prM
           537 PRM
L16          0 L15 AND PRM

=> s l15 and M
        369387 M
L17          2 L15 AND M

=> d l17,cbib,ab,1-2

L17  ANSWER 1 OF 2     MEDLINE on STN
1998161568  Document Number: 98161568.     PubMed ID: 9502462.   Etiology of
     acute childhood encephalitis at The Hospital for Sick Children, Toronto,
     1994-1995. Kolski H; Ford-Jones E L; Richardson S; Petric M; Nelson S;
     Jamieson F; Blaser S; Gold R; Otsubo H; Heurter H; MacGregor D.
     (Department of Pediatrics, The Hospital for Sick Children and the
     University of Toronto, Ontario, Canada. ) CLINICAL INFECTIOUS DISEASES,
     (1998 Feb) 26 (2) 398-409.  Journal code: 9203213. ISSN: 1058-4838. Pub.
     country: United States. Language: English.
AB   Of 145 patients admitted to our hospital because of encephalitis-like
     illness, 50 patients hospitalized for > or =72 hours underwent
     standardized microbiological investigations.  A confirmed or probable
     etiologic agent was identified in 20 cases (40%), including Mycoplasma
     pneumoniae (9 cases).  M. pneumoniae and enterovirus (2), herpes simplex
     virus (4), Epstein-Barr virus (1), human herpes-virus 6 (HHV-6) (1), HHV-6
     and influenza virus type A (1), influenza virus type A (1), and Powassan
     virus (1).  In 13 cases (26%), a possible pathogen was identified,
     including M. pneumoniae in nine cases.  Presenting features included
     fever (80% of patients), seizures (78%), focal neurological findings
     (78%), and decreased consciousness (47%).  The frequency of findings at
     the time of admission vs. later in hospitalization was as follows:
     pleocytosis, 59% vs. 63%; electroencephalogram abnormalities, 87% vs. 96%;
     and neuroimaging abnormalities, 37% vs. 69%, respectively.  The outcomes
     at the time of discharge were as follows: normal results of physical
     examination, 32% (16) of the patients; death, 2% (1); motor difficulties,
     26% (13); global neurological deficits, 16% (severe, 6; mild, 2); mental
     status changes, 14% (7); visual defects, 8% (4); and hearing impairment,
     2% (1).

L17  ANSWER 2 OF 2     MEDLINE on STN
72204845  Document Number: 72204845.     PubMed ID: 4624393.   Dose-dependent
     viremia and the differential immunoglobulin response of hamsters to
     Powassan virus. Chernesky M A; Whittaker-Haines P J. CANADIAN JOURNAL
     OF MICROBIOLOGY, (1972 May) 18 (5) 655-61.  Journal code: 0372707. ISSN:
     0008-4166. Pub. country: Canada. Language: English.


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     (FILE 'HOME' ENTERED AT 15:35:18 ON 23 FEB 2004)

     FILE 'USPATFULL' ENTERED AT 15:35:57 ON 23 FEB 2004
L1          661 S YELLOW FEVER VIRUS
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L3           17 S L2 AND (PRM AND E)
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L7          231 S ST. LOUIS ENCEPHALITIS VIRUS
L8           16 S L7 AND LOUIS/CLM
L9           13 S L8 NOT L5
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L10          52 S POWASSAN VIRUS
L11           6 S L10 AND POWASSAN/CLM
L12           2 S L11 NOT L9
L13         264 S WEST NILE VIRUS
L14          34 S L13 AND (WEST NILE VIRUS/CLM)

     FILE 'MEDLINE' ENTERED AT 16:30:55 ON 23 FEB 2004
L15          65 S POWASSAN VIRUS
L16           0 S L15 AND PRM
L17           2 S L15 AND M

=> d l15,ti,40-65

L15  ANSWER 40 OF 65     MEDLINE on STN
TI   [Electron microscopic study of the An-750 strain of Powassan virus
     isolated in the Soviet Union].
     Elektronno-mikroskopicheskoe issledovanie shtamma An-750 virusa Povassan,
     vydelennogo na territorii Sovetskogo Soiuza.

L15  ANSWER 41 OF 65     MEDLINE on STN
TI   [Neural pathway of Powassan virus spread in the central nervous system
     of white mice].
     Nevral'nyi put' rasprostraneniia virusa Povassan v tsentral'noi nervnoi
     sisteme belykh myshei.

L15  ANSWER 42 OF 65     MEDLINE on STN
TI   [Pathomorphology of experimental infection caused by powassan virus
     isolated in the Primorskii Territory].
     Patomorfologiia eksperimental'noi infektsii, vyzvannoi virusom Povassan,
     vydelennym v Primorskom Krae.

L15  ANSWER 43 OF 65     MEDLINE on STN
TI   Experimental milk-borne transmission of Powassan virus in the goat.

L15  ANSWER 44 OF 65     MEDLINE on STN
TI   [Isolation of the Powassan virus from Haemaphysalis neumanni Donitz,
     1905 ticks in the Maritime Territory].
     Izoliatsiia virusa Povassan iz kleshchei Haemaphysalis neumanni Donitz,
     1905, v Primorskom krae.

L15  ANSWER 45 OF 65     MEDLINE on STN
TI   A case of Powassan virus encephalitis.

L15  ANSWER 46 OF 65     MEDLINE on STN
TI   Powassan virus infection. A report of three human cases of encephalitis.

L15  ANSWER 47 OF 65     MEDLINE on STN
TI   A non-fatal human case of Powassan virus encephalitis.

L15  ANSWER 48 OF 65     MEDLINE on STN
TI   Immunization of mice against Russian spring-summer virus complex and
     monkeys against Powassan virus with attenuated Langat E5 virus.
     Duration of protection.

L15  ANSWER 49 OF 65     MEDLINE on STN
TI   Dose-dependent viremia and the differential immunoglobulin response of
     hamsters to Powassan virus.

L15  ANSWER 50 OF 65     MEDLINE on STN
TI   Powassan virus infection in the grey squirrel.

L15  ANSWER 51 OF 65     MEDLINE on STN
TI   California encephalitis and Powassan virus activity in British
     Columbia, 1969.

L15  ANSWER 52 OF 65     MEDLINE on STN
TI   Localization of Powassan virus in Dermacentor andersoni ticks by
     immunofluorescence.

L15  ANSWER 53 OF 65     MEDLINE on STN
TI   Powassan virus transmission by ixodid ticks infected after feeding on
     viremic rabbits injected intravenously.

L15  ANSWER 54 OF 65     MEDLINE on STN
TI   Westward extension of Powassan virus prevalence.

L15  ANSWER 55 OF 65     MEDLINE on STN
TI   Powassan virus: persistence of virus activity during 1966.
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L15 ANSWER 56 OF 65 MEDLINE on STN
TI A chick embryo attenuated strain (TP21 E5) of Langat virus. 3. The ability to protect against homologous virus and **Powassan virus** in cross-challenge experiments.

L15 ANSWER 57 OF 65 MEDLINE on STN
TI **Powassan virus**: vernal spread during 1965.

L15 ANSWER 58 OF 65 MEDLINE on STN
TI Interferon production in **Powassan virus** infections of chickens.

L15 ANSWER 59 OF 65 MEDLINE on STN
TI THE FIRST ISOLATIONS OF **POWASSAN VIRUS** IN NEW YORK STATE.

L15 ANSWER 60 OF 65 MEDLINE on STN
TI **POWASSAN VIRUS**: SUMMER INFECTION CYCLE, 1964.

L15 ANSWER 61 OF 65 MEDLINE on STN
TI **POWASSAN VIRUS**: FIELD INVESTIGATIONS DURING THE SUMMER OF 1963.

L15 ANSWER 62 OF 65 MEDLINE on STN
TI **POWASSAN VIRUS**: MORPHOLOGY AND CYTOPATHOLOGY.

L15 ANSWER 63 OF 65 MEDLINE on STN
TI Isolation of a virus closely related to **Powassan virus** from Dermacentor andersoni collected along North Cache la Poudre River, Colo.

L15 ANSWER 64 OF 65 MEDLINE on STN
TI **Powassan virus**: investigations of possible natural cycles of infection.

L15 ANSWER 65 OF 65 MEDLINE on STN
TI **Powassan virus**: isolation of virus from a fatal case of encephalitis.

=> d l15,ti,20-39

L15 ANSWER 20 OF 65 MEDLINE on STN
TI Agents of equine viral encephalomyelitis: correlation of serum and cerebrospinal fluid antibodies.

L15 ANSWER 21 OF 65 MEDLINE on STN
TI Evidence that the mature form of the flavivirus nonstructural protein NS1 is a dimer.

L15 ANSWER 22 OF 65 MEDLINE on STN
TI California serogroup and **Powassan virus** infection of cats.

L15 ANSWER 23 OF 65 MEDLINE on STN
TI Isolation of **Powassan virus** from a spotted skunk in California.

L15 ANSWER 24 OF 65 MEDLINE on STN
TI Two kinds of monoclonal antibodies to tick-borne encephalitis virus.

L15 ANSWER 25 OF 65 MEDLINE on STN
TI Variability of **Powassan virus** cultured in tissue explants and organism of Hyalomma anatolicum ticks.

L15 ANSWER 26 OF 65 MEDLINE on STN
TI Powassan viral encephalitis: a review and experimental studies in the horse and rabbit.

L15 ANSWER 27 OF 65 MEDLINE on STN
TI Experimental encephalitis in monkeys caused by the **Powassan virus**.

L15 ANSWER 28 OF 65 MEDLINE on STN
TI [Electrophoretic characteristics of the virus-specific proteins of **Powassan virus**].
Osobennosti elektroforeticheskikh kharakteristik virusspetsificheskikh belkov virusa Povassan.

L15 ANSWER 29 OF 65 MEDLINE on STN
TI [Comparative analysis of the electrophoretic mobility of the high-molecular virus-specific proteins of flaviviruses].
Sravnitel'nyi analiz elektroforeticheskoi podvizhnosti vysoko-molekuliarnykh virusspetsificheskikh belkov flavivirusov.

L15 ANSWER 30 OF 65 MEDLINE on STN
TI Isolation of Francisella tularensis and **Powassan virus** from ticks (Acari: Ixodidae) in Ontario, Canada.

L15 ANSWER 31 OF 65 MEDLINE on STN
TI Antigenic relationships among viruses of the tick-borne encephalitis complex as studied by monoclonal antibodies.

L15 ANSWER 32 OF 65 MEDLINE on STN
TI **Powassan virus** encephalitis resembling herpes simplex encephalitis.

L15 ANSWER 33 OF 65 MEDLINE on STN
TI **Powassan virus** infection in snowshoe hares (Lepus americanus).

L15 ANSWER 34 OF 65 MEDLINE on STN
TI [Experimental monkey encephalitis caused by **Powassan virus**].
Ob eksperimental'nom entsefalite obez'ian, vyzvannom virusom Povassan.

L15 ANSWER 35 OF 65 MEDLINE on STN
TI **Powassan virus** encephalitis in southeastern Ontario.

L15 ANSWER 36 OF 65 MEDLINE on STN
TI [Role of **Powassan virus** in the etiological structure of tick-borne encephalitis in the Primorsky Kray].
Izuchenie roli virusa Povassan v etiologicheskoi strukture kleshchevogo entsefalita v Primorskom krae.

L15 ANSWER 37 OF 65 MEDLINE on STN
TI **Powassan virus** in Ixodes cookei and Mustelidae in New England.

L15 ANSWER 38 OF 65 MEDLINE on STN
TI **Powassan virus** meningoencephalitis: a case report.

L15 ANSWER 39 OF 65 MEDLINE on STN
TI Light and electron microscope study of the neurotropism of **Powassan virus** strain P-40.

=> d l15,ti,1-19

L15 ANSWER 1 OF 65 MEDLINE on STN
TI Tick-borne flaviviruses.

L15 ANSWER 2 OF 65 MEDLINE on STN
TI The importance of the Q motif in the ATPase activity of a viral helicase.

L15 ANSWER 3 OF 65 MEDLINE on STN
TI Viral meningitis and encephalitis: traditional and emerging viral agents.

L15 ANSWER 4 OF 65 MEDLINE on STN
TI Tick-borne encephalitis.

L15 ANSWER 5 OF 65 MEDLINE on STN
TI Human surveillance for West Nile virus infection in Ontario in 2000.

L15 ANSWER 6 OF 65 MEDLINE on STN
TI Nucleotide sequencing and serological evidence that the recently recognized deer tick virus is a genotype of **Powassan virus**.

L15 ANSWER 7 OF 65 MEDLINE on STN
TI Phylogeny of North American **Powassan virus**.

L15 ANSWER 8 OF 65 MEDLINE on STN
TI Etiology of acute childhood encephalitis at The Hospital for Sick Children, Toronto, 1994-1995.

L15 ANSWER 9 OF 65 MEDLINE on STN
TI A new tick-borne encephalitis-like virus infecting New England deer ticks, Ixodes dammini.

L15 ANSWER 10 OF 65 MEDLINE on STN
TI Experimental transmission of **Powassan virus** (Flaviviridae) by Ixodes scapularis ticks (Acari:Ixodidae).

L15 ANSWER 11 OF 65 MEDLINE on STN
TI [Biological properties and antigenic interconnection between Tiuleniy and Karshi flaviviruses].
Biologicheskie svoistva i antigennye vzaimosviazi flavivirusov tiulenii i karshi.

L15 ANSWER 12 OF 65 MEDLINE on STN
TI [Genome sequence and antigen structure of the **Powassan virus**: analysis

of genetic elements of tick-transmitted flaviviruses].
Genomsequenz und Antigenstruktur des **Powassan-Virus**: Analyse genetischer Elemente von durch Zecken ubertragenen Flaviviren.

L15 ANSWER 13 OF 65 MEDLINE on STN
TI Classification of a new member of the TBE flavivirus subgroup by its immunological, pathogenetic and molecular characteristics: identification of subgroup-specific pentapeptides.

L15 ANSWER 14 OF 65 MEDLINE on STN
TI Complete genomic sequence of **Powassan virus**: evaluation of genetic elements in tick-borne versus mosquito-borne flaviviruses.

L15 ANSWER 15 OF 65 MEDLINE on STN
TI [The specific reactivity of cDNA and deoxyoligonucleotide probes, complementary to the tick-borne encephalitis virus genome, with the RNA of strains of different geographical origins].
Spetsificheskaia reaktivnost' kDNK- i dezoksioligonukleotidnykh zondov, komplementarnykh genomu virusa kleshchevogo entsefalita, s RNK shtammov razlichnogo geograficheskogo proiskhozhdeniia.

L15 ANSWER 16 OF 65 MEDLINE on STN
TI [The differentiation of viruses of the tick-borne encephalitis complex by means of RNA-DNA hybridization].
Differentsiatsiia virusov kompleksa kleshchevogo entsefalita metodom RNK--DNK-gibridizatsii.

L15 ANSWER 17 OF 65 MEDLINE on STN
TI Differentiation of strains of tick-borne encephalitis virus by means of RNA-DNA hybridization.

L15 ANSWER 18 OF 65 MEDLINE on STN
TI [The significance of Ixodes ticks in the southern Far East in the circulation of **Powassan virus**].
Znachenie iksodovykh kleshchei ioga dal'nego vostoka v tsirkuliatsii virusa povassan.

L15 ANSWER 19 OF 65 MEDLINE on STN
TI Leg weakness associated with **Powassan virus** infection--Ontario.

=> d l15,cbib,ab,7,14

L15 ANSWER 7 OF 65 MEDLINE on STN
2001349846 Document Number: 21306199. PubMed ID: 11413377. Phylogeny of North American **Powassan virus**. Ebel G D; Spielman A; Telford S R 3rd. (Laboratory of Public Health Entomology, Department of Immunology and Infectious Diseases, Harvard School of Public Health, 665 Huntington Ave, Boston, MA 02115, USA.. ebel@wadsworth.org) . JOURNAL OF GENERAL VIROLOGY, (2001 Jul) 82 (Pt 7) 1657-65. Journal code: 0077340. ISSN: 0022-1317. Pub. country: England: United Kingdom. Language: English.
AB To determine whether **Powassan virus** (POW) and deer tick virus (DTV) constitute distinct flaviviral populations transmitted by ixodid ticks in North America, we analysed diverse nucleotide sequences from 16 strains of these viruses. Two distinct genetic lineages are evident, which may be defined by geographical and host associations. The nucleotide and amino acid sequences of lineage one (comprising New York and Canadian POW isolates) are highly conserved across time and space, but those of lineage two (comprising isolates from deer ticks and a fox) are more variable. The divergence between lineages is much greater than the variation within either lineage, and lineage two appears to be more diverse genetically than is lineage one. Application of McDonald-Kreitman tests to the sequences of these strains indicates that adaptive evolution of the envelope protein separates lineage one from lineage two. The two POW lineages circulating in North America possess a pattern of genetic diversity suggesting that they comprise distinct subtypes that may perpetuate in separate enzootic cycles.

L15 ANSWER 14 OF 65 MEDLINE on STN
93242744 Document Number: 93242744. PubMed ID: 8097605. Complete genomic sequence of **Powassan virus**: evaluation of genetic elements in tick-borne versus mosquito-borne flaviviruses. Mandl C W; Holzmann H; Kunz C; Heinz F X. (Institute of Virology, Vienna, Austria.) VIROLOGY, (1993 May) 194 (1) 173-84. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.
AB The complete nucleotide sequence of the positive-stranded RNA genome of the tick-borne flavivirus Powassan (10,839 nucleotides) was elucidated and the amino acid sequence of all viral proteins was derived. Based on this sequence as well as serological data, **Powassan virus** represents the

most divergent member of the tick-borne serocomplex within the genus flaviviruses, family Flaviviridae. The primary nucleotide sequence and potential RNA secondary structures of the **Powassan virus** genome as well as the protein sequences and the reactivities of the virion with a panel of monoclonal antibodies were compared to other tick-borne and mosquito-borne flaviviruses. These analyses corroborated significant differences between tick-borne and mosquito-borne flaviviruses, but also emphasized structural elements that are conserved among both vector groups. The comparisons among tick-borne flaviviruses revealed conserved sequence elements that might represent important determinants of the tick-borne flavivirus phenotype.

=> d his

(FILE 'HOME' ENTERED AT 15:35:18 ON 23 FEB 2004)

FILE 'USPATFULL' ENTERED AT 15:35:57 ON 23 FEB 2004

L1	661	S	YELLOW FEVER VIRUS
L2	59	S	L1 AND (YELLOW FEVER VIRUS/CLM)
L3	17	S	L2 AND (PRM AND E)
L4	4	S	L3 AND (PRM/CLM)
L5	13	S	L3 NOT L4
L6	7	S	L3 AND E/CLM
L7	231	S	ST. LOUIS ENCEPHALITIS VIRUS
L8	16	S	L7 AND LOUIS/CLM
L9	13	S	L8 NOT L5
L10	52	S	POWASSAN VIRUS
L11	6	S	L10 AND POWASSAN/CLM
L12	2	S	L11 NOT L9
L13	264	S	WEST NILE VIRUS
L14	34	S	L13 AND (WEST NILE VIRUS/CLM)

FILE 'MEDLINE' ENTERED AT 16:30:55 ON 23 FEB 2004

L15	65	S	POWASSAN VIRUS
L16	0	S	L15 AND PRM
L17	2	S	L15 AND M

=> log off
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
STN INTERNATIONAL LOGOFF AT 16:46:46 ON 23 FEB 2004